Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits *in vivo*.

Ira Espuny-Camacho[1], Kimmo A. Michelsen[1], David Gall[2], Daniele Linaro[3], Anja Hasche[1], Jérôme Bonnefont[1], Camilia Bali[1], David Orduz[2], Nelle Lambert[1], Nicolas Gaspard[1], Sophie Péron[4], Serge N. Schiffmann[2], Michele Giugliano[3], Afsaneh Gaillard[4], Pierre Vanderhaeghen[1,]

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[4] INSERM, Experimental and Clinical Neurosciences Laboratory, Universite de Poitiers, France.

Keywords:
human pluripotent stem cells
cortical differentiation
temporal patterning
in vivo transplantation and integration into the host
axogenesis and neuronal maturation

Background and novelty

The cerebral cortex is the most complex structure of our brain. During evolution, the relative size of the cortex has increased considerably among higher mammals and new cortical areas involved in higher evolved functions have emerged. The study of human cortical development has major

implications for brain evolution and cortical related diseases, but has remained elusive due to paucity of experimental models.
Here, we describe an intrinsic pathway of corticogenesis from human embryonic (ESC) and induced pluripotent (iPSC) stem cells leading to the sequential generation of first forebrain progenitors and later pyramidal neurons of all six layers identities in a time-dependent fashion, highly reminiscent of the in vivo situation.

Experimental approach

We describe an in vitro model for the directed differentiation of human pluripotent stem cells in a monolayer fashion and devoided of morphogens, but supplemented with noggin, and inhibitor of the BMP pathway, that has been shown to be required for neuroectoderm specification. Specified progenitors and neurons are later transplanted into mouse newborn brain and analysed after several months in vivo by immunofluorescence analysis and by patch-clamp recordings.

Results and discussion

Following the in vitro differentiation, human pluripotent stem cells efficiently differentiated into forebrain and telencephalic progenitors based on the expression of various genes tested by immunofluorescence, quantitative PCR and microarray analysis. At later stages, these cells exited cell cycle and became cortical pyramidal neurons, as attested by their pyramidal morphology, but also by the expression of various markers of cortical neurons and cortical layer specific genes. The cortical neurons present markers of connectivity and a mature electrophisiological profile at later stages. Moreover, following grafting into the mouse newborn cortex, the human ESC-derived neurons extended axons to endogenous cortical targets, present numerous synapses and functionally integrated into the host.

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European Society for Animal Cell Technology

23rd ESACT meeting

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Dear Colleagues and Participants of the 23rd ESACT General Meeting,

On behalf of the Organising and Scientific Committees of the 23rd ESACT Conference and Exhibition, as well as on behalf of the Executive Committee of ESACT, I am very pleased to welcome you to Lille, the 4th largest French metropolitan area, an artistic and historic city, but also an important center of economy, higher education and R&D!

This ESACT Meeting will be focused on "Better Cells for Better Health" highlighting both the importance of basic aspects and high-performing bioprocesses, as well as recent advances in stem cells, recombinant proteins and viral vectors. The scientific programme includes keynote and invited lectures, oral communications, posters and workshops. The traditional meeting format, by now a distinguishing feature of ESACT Meetings, is designed to promote scientific and technical interactions among participants, as well as networking: No parallel oral sessions, an unparalleled industrial exhibition featuring the latest technologies, systems and services in animal cell technology, a very rich poster programme and a large variety of social events.

The organization of an ESACT Meeting is an honour, but also a very complex undertaking, which involves a lot of planning and hard work. I would like to thank cordially all those who made possible the organisation of this major event:

- All the members of the Organising Committee who diligently took care of all the practical aspects, scientific and social ones, and I obviously include here the team of Le Public Système (LPS), our PCO, for the excellent, timely and professional support.
- The members of the Scientific Committee who defined the scientific content and selected abstracts and posters, including the team who will be in charge of the poster selection process for the Poster Prize.
- The members of the ESACT Executive Committee for their invaluable support all along the process.

We all cordially look forward to meeting old friends, introducing newcomers to the area of animal cell technology and experiencing lively interactions and discussions on the newest hot topics in the field.

I am sure that your stay in Lille will be an enjoyable and memorable one.

Enjoy the science, the flair and the social program!

Yves-Jacques Schneider
Chairman of the 23rd ESACT Meeting
Full Professor of Biochemistry & Chairman of the Life Science Institute
University of Louvain (UCL), Louvain-la-Neuve Belgium

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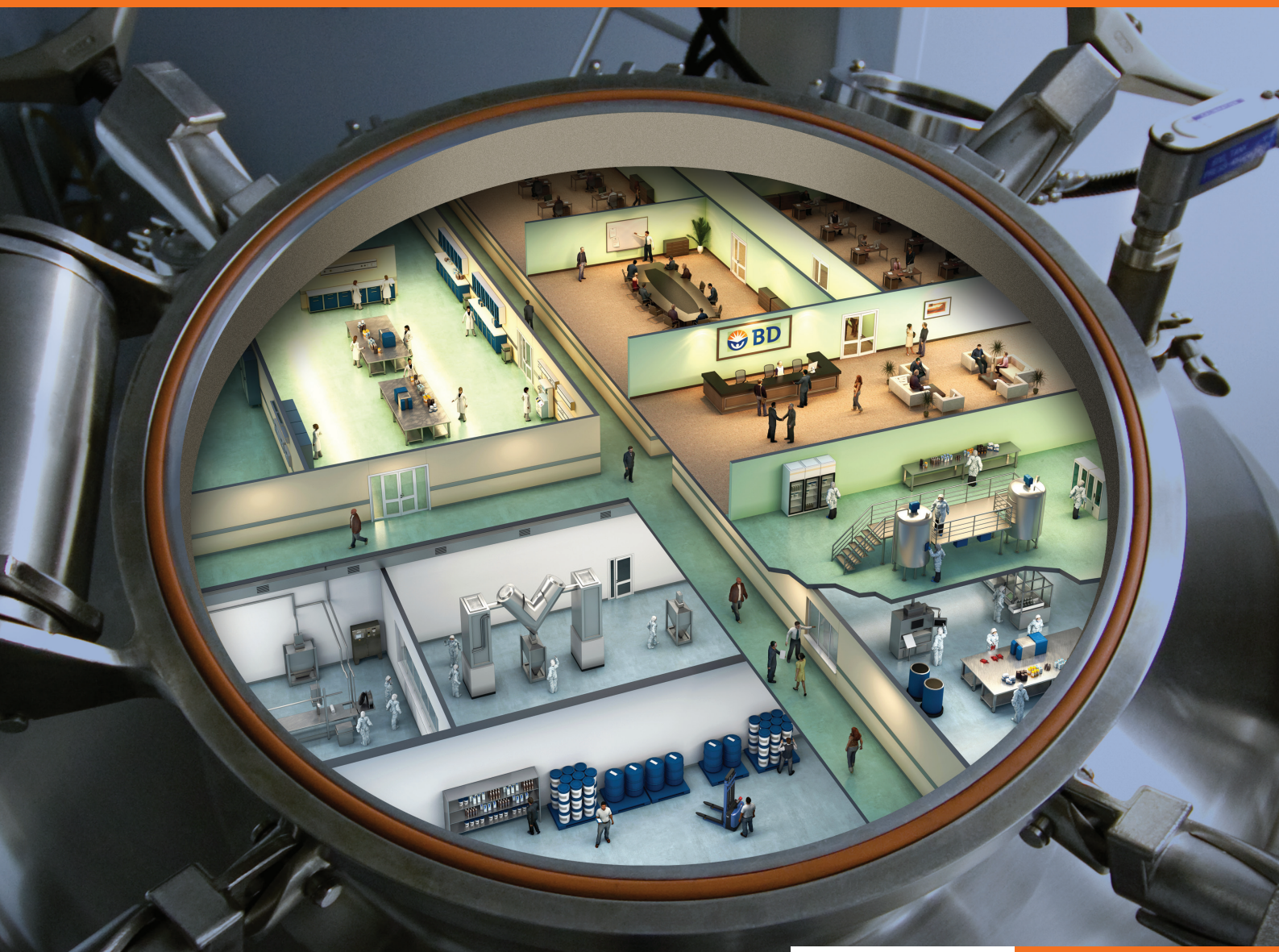

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7 Loveton Circle
Sparks, MD 21152
bdbiosciences.com

Congress Venue

Lille Grand Palais
1, Boulevard des Cites-Unies
59777 Euralille – France
Metro: Line 2 – Station: LILLE GRAND PALAIS

Opening Hours

	The Welcome Desk Level 1	Exhibition Area Level 3	Hot Line
Saturday 22nd of June	15h00 / 19h00		+33 (0)3 20 14 14 50
Sunday 23rd of June	8h00 / 21h00	14h00 / 21h00	
Monday 24th of June	8h00 / 19h00	8h00 / 19h00	
Tuesday 25th of June	8h00 / 15h30	8h00 / 15h00	
Wednesday 26th of June	8h00 / 19h00	8h00 / 17h00	

Wifi Access

Wifi access: esactmeeting
Password: lille2013

Access to the Congress

The delegate fees give access to the following:

- Access to all the conferences of the ESACT Congress
- ESACT Congress Lunches
- Traders Cocktail on Sunday, June 23rd
- Excursion on Tuesday, June 25th 2013 afternoon (pre-registration compulsory)
- Excursion Dinner on Tuesday, June 25th
- Gala Dinner on Wednesday, June 26th

Accompanying person fees give access to:

- Congress Lunches
- Excursion
- Excursion Dinner
- Gala Dinner

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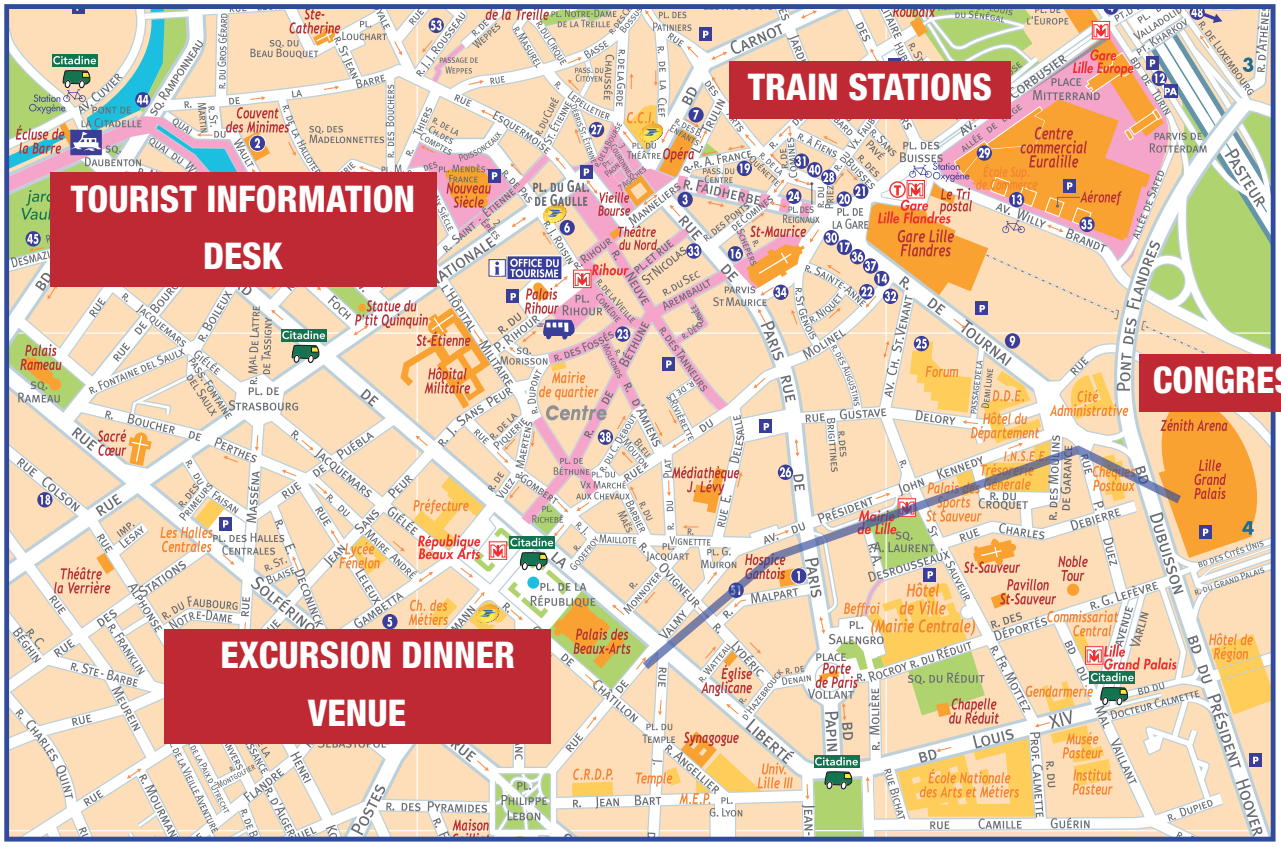
Metro map

Lille Map



Access to Lille Museum of Fine Arts: 15 min walking distance from Lille Grand Palais

Excursions Conditions

One ticket per participant is included in the registration fees. The number of participants is limited for some excursions and strict first-come-first-served order will be observed. No change in excursion choice is possible once registration is made. This excursion is offered by the Organising Committee. There will be no refund if you choose not to attend.

Bus Schedule for Excursions

	Departure from Lille Grand Palais	Return
TOUR 1: LILLE & The Royal district and Charles de Gaulle Birthplace	15h00	The cocktail-dinner will take place at Lille Museum of Fine Arts (see the map above). You can access by walking from Lille Grand Palais. A bus will be at your disposal at 19h00 in front of Lille Grand Palais with a limited number of seats.
TOUR 2: LILLE & The « Dutch gin » trail	14h00	
TOUR 3: LILLE & the LaM	15h00	
TOUR 4: LILLE & The Old Disctrict	15h00	
TOUR 5: BRUGES	14h30	The buses will bring you directly to Lille Museum of Fine Art
TOUR 6: GHENT	14h30	
TOUR 7: ARRAS & The Wellington Quarry	15h00	
TOUR 8: LEWARDE: The mining history	14h30	
TOUR 9: LILLE BY BUS	15h00	

Connecting with patients

UCB has a passionate, long-term commitment to finding more effective treatments for several specific diseases in the central nervous system and immunology disorders. Our challenge is to help patients and families living with the physical and social burden of severe diseases. It holds out the promise of a new generation of therapies that will enable them to enjoy more normal, everyday lives.

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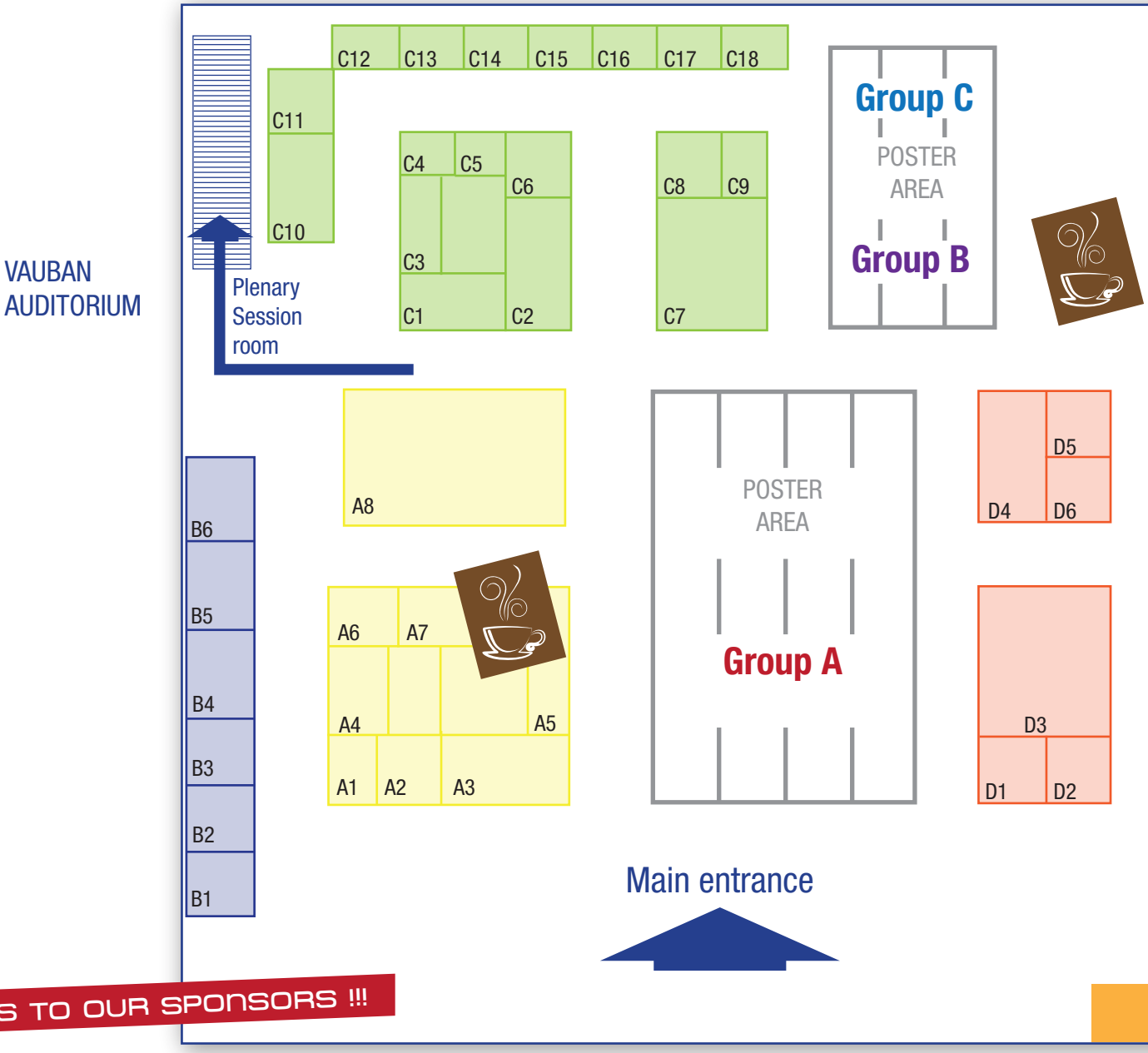

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BLUESENS GAS SENSOR GMBH	F8	FRAUNHOFER ITEM	G2
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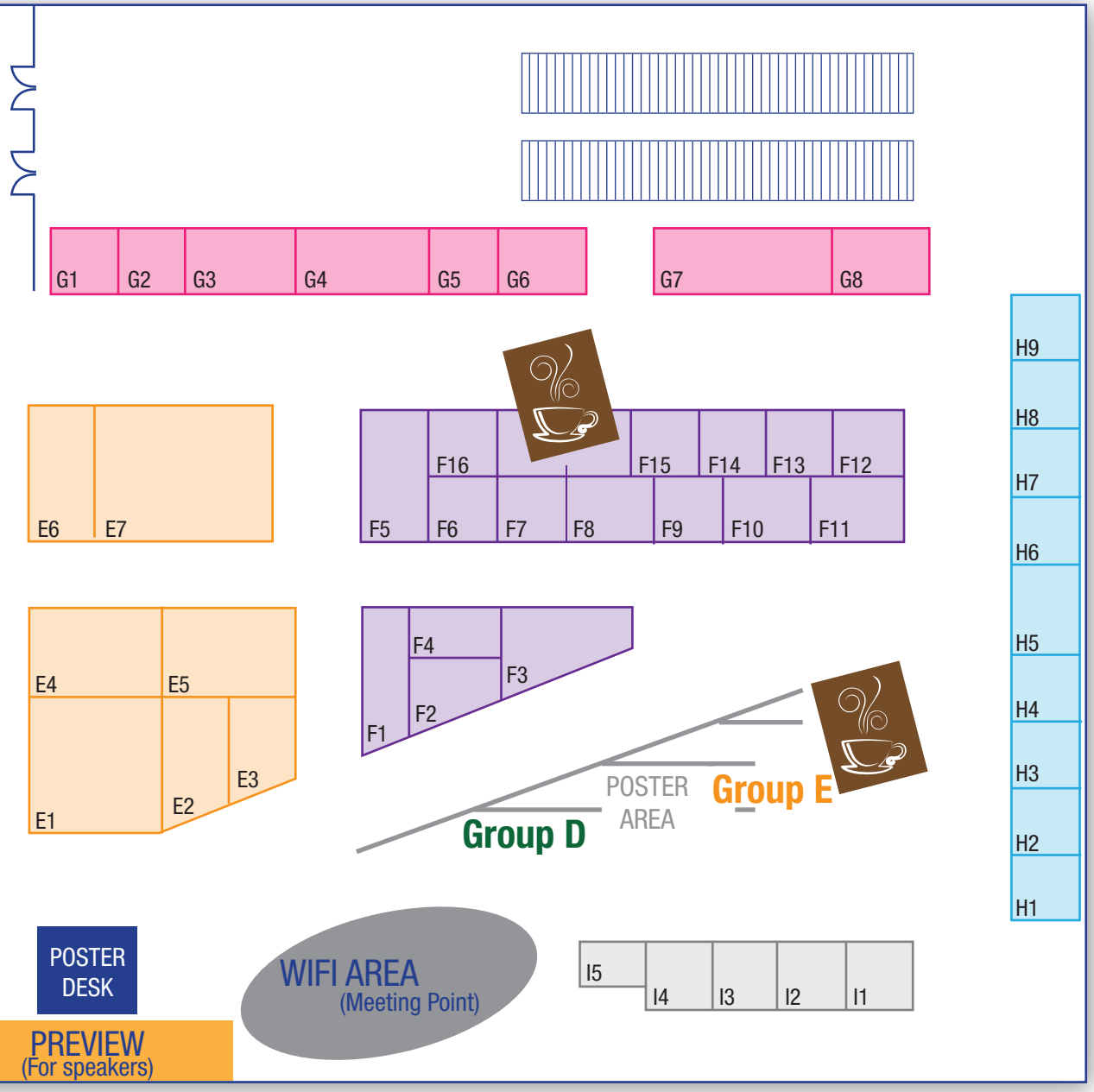
CELLECTIS BIORESEARCH	I4	GENETIC ENGINEERING & BIOTECHNOLOGY NEWS	A1
CELLON SA	G6		



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HAMILTON BONADUZ AG	F2	PNEUMATIC SCALES ANGELUS - CARR CENTRITECH	A4
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INSILICO BIOTECHNOLOGY AG	F14	PRESENS PRECISION SENSING GMBH	F1
IRVINE SCIENTIFIC	G4	PROTAGEN PROTEIN SERVICES GMBH	B2
ISBIO	C4	PROTEIGENE	I2
IUL INSTRUMENTS GMBH	H6	RD-BIOTECH	C9
JM BIOCONNECT	C1	REFINE TECHNOLOGIES	G3
KAISER OPTICAL SYSTEMS	F7	RENTSCHLER BIOTECHNOLOGIE GMBH	H4
KERRY INC.	C2	REPLIGEN	A2
LGC STANDARDS	D6	SAFC AND BIORELIANCE	A3
LONZA	E1	SAINT-GOBAIN PERFORMANCE PLASTICS	B1
MAXCYTE INC.	C8	SARTORIUS STEDIM BIOTECH GMBH	D3
MEDICYTE GMBH	C15	SGS VITROLOGY	G8
MERCK MILLIPORE	G7	SPECTRUM LABORATORIES INC	F4
METABOLON INC	E2	SPINNOVATION ANALYTICAL B.V	I5
MILTENYI BIOTEC	A6	TAP BIOSYSTEMS	H2
MOLECULAR DEVICES	F16	THERMO SCIENTIFIC	E7
NOVA BIOMEDICAL	F11	VIRUSURE GMBH	C6
OPTOCELL TECHNOLOGY	H5	WU XI APP TEC	F13

SUNDAY 23RD OF JUNE

	Rooms	EUROTOP Level 5	LIEGE Level 1	VAN GOGH Level 8	MATISSE Level 8	TURIN Level 1	ARTOIS Level 5
INDUSTRIAL	8h00	Doors opening					
	9h00	Thermo SCIENTIFIC	GE Healthcare	BD		ATMI	IrvineScientific
	11h00	Coffee Break in the Hall in front of each Workshop room					
	11h15	Thermo SCIENTIFIC	sartorius stedim	BD	Boehringer Ingelheim	KERRY	SAFC
	13h15	Lunch Boxes offered in the Hall in front of each Workshop room					
TOPICAL	13h45	Therapeutic vaccines	Metabolism as a key for improvement of cell culture processes - status and advances in analytics and data analysis	Is tomorrow's process fed-batch or perfusion?	The www.CHOgenome.org Resource for the International CHO Biotechnology Community		
	15h45	End of Workshop session					

SUNDAY 23RD OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
16h00	Opening Ceremony	Exhibition & Posters
16h30	Keynote Lecture: A. CAPLAN	
17h15	SESSION I: CELL THERAPY AND VACCINES	
18h35	End of the session	
19h00 to 21h00	TRADERS RECEPTION in the EXHIBITION AREA	

MONDAY 24TH OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
8h00	Doors opening	
9h00	SESSION II: ADVANCED CELLULAR MODELS	Exhibition & Posters
10h30	Coffee Break	
11h00	SESSION II: ADVANCED CELLULAR MODELS	Exhibition & Posters
12h30	Lunch Break in Jeanne de Flandre room	POSTERS SESSION A
14h30	SESSION III: EPIGENETICS AND SYNTHETIC BIOLOGY	Exhibition & Posters
16h30	Coffee Break	
17h00	SESSION III: EPIGENETICS AND SYNTHETIC BIOLOGY	Exhibition & Posters
18h10	END OF THE DAY	

TUESDAY 25TH OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
8h00	Doors opening	
9h00	SESSION IV: GENETIC AND PROCESS ENGINEERING	Exhibition & Posters
10h30	Coffee Break	
11h00	SESSION IV: GENETIC AND PROCESS ENGINEERING	Exhibition & Posters
12h30	Lunch Break in Jeanne de Flandre room	POSTERS SESSION B
15h00	END OF THE DAY	
15h00 to 20h00	OUTING & Cocktail-Dinner at the Museum of Fine Arts of Lille	

WEDNESDAY 26TH OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
8h00	Doors opening	
9h00	SESSION V: NEXT GENERATION MOLECULE FORMATS	Exhibition & Posters
10h30	Coffee Break	
11h00	SESSION VI: CELL INTERACTIONS	Exhibition & Posters
12h30	Lunch Break in Jeanne de Flandre room	POSTERS SESSION C
14h30	Keynote Lecture: C. VERFAILLIE	Exhibition & Posters
15h15	SHORT POSTER PRESENTATIONS & POSTER PRIZE SELECTION	
17h15	Closing Ceremony	
17h30	END OF THE DAY	
19h00	GALA DINNER at Farmhouse of the Templars	

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PROGRAMME OF SUNDAY 23RD OF JUNE
INDUSTRIAL WORKSHOPS

TURIN ROOM LEVEL 1

ATMI

From 9h00 to 11h00

Single-Use Technologies to Fast Track Human Vaccine Production, from Development to Industrial Manufacturing.
Illustrated by a case study related to a new rabies vaccine production, this workshop will address the implementation of single-use technologies to fast track the process. From development to industrial scale, presentations will highlight key decision factors, challenges faced during implementation and current results achieved. Chaired by Jean-Marc Guillaume USP Director, Bioprocess R&D from Sanofi Pasteur, you will discover the perspectives from an industrial stakeholder on the lessons learned from single-use technologies. Conclusions will include insight from the technological partner and the organizational impact from supporting and meeting quality and supply chain requirements of a key partner.

Chairman: *Jean-Marc Guillaume, USP Director, Bioprocess R&D, Sanofi Pasteur*
Speakers: *Jean-Marc Guillaume, USP Director, Bioprocess R&D, Sanofi Pasteur*
Nicolas Seve, Process Scientist, USP, Bioprocess R&D, Sanofi Pasteur
Eric Calvosa, Process Manager, USP, Bioprocess R&D, Sanofi Pasteur
Ahmed Farouk, Single-Use Bioreactor Specialist, Application Lab engineer
Sven Adams, Supply Chain & Purchasing Manager

VAN GOGH ROOM LEVEL 8

BD

From 9h00 to 11h00

Workshop 1:
1. Cell Culture Media and Process Optimization: Diverse Challenges and Optimal Solutions
James W. Brooks, PhD, R&D Manager, BD Biosciences – Advanced Bioprocessing
Diverse cell types utilized in bioprocessing and cell therapy have unique nutritional requirements. To achieve maximal cell performance, the optimal cell culture medium, supplementation, and process are required. We present a multi-faceted approach to media and process optimization, along with case studies demonstrating achieved performance improvement.

2. Rapid Media Design – The Chemically Defined Medium Platform
Karlheinz Landauer, PhD, COO, Celonic
With more than 15 years of experience in process development, Celonic has designed a chemically defined medium platform for the bioprocessing industry. This platform consists of a basal medium composition and a universal feeding solution for virtually all basal media, which is manufactured in BD's state of the art, fully-dedicated animal-free media production facility. This workshop will be presenting case studies in which Celonic tested the platform on several host cell lines and production cell lines in different laboratories around the world.

Workshop 2: From 11h15 to 13h15
The Path to Chemically-Defined Bioprocessing
Elizabeth C. Dodson, PhD, R&D Manager, BD Biosciences – Advanced Bioprocessing
In biopharmaceutical production, optimization of cell culture parameters is a central component of process development. One challenge facing process development scientists is the initial selection of an appropriate cell culture supplement or feed that will give desired titer, growth characteristics, and protein quality. Each biopharmaceutical process requires a unique cell culture environment for optimized performance. The availability of a family of chemically defined (CD) supplements offering a diverse performance profile can significantly improve process development timelines. To facilitate process development, BD will commercialize a diverse set of CD supplements. In this workshop we will share our findings that were made during the discovery process.
- How can you utilize DOEs to facilitate development and optimization of a CD supplement?
- How do you select a base medium for optimal performance with a CD supplement?
- How do you optimize a feeding regimen for top performance with a CD supplement?
- How do you scale up from shake flask to bioreactor to obtain optimal CD supplement performance?
- How do your CD supplements influence protein glycosylation patterns?

MATISSE ROOM LEVEL 8

Boehringer Ingelheim

From 11h15 to 13h15

Producing Value from Discovery to Supply for the Next Generation of Biologics
- Company presentation BI
Stephan Schlenker, Boehringer Ingelheim
- Speed, flexibility in process development
Benedikt Greulich, Boehringer Ingelheim
- Fit for pipeline concepts for antibody discovery and development
Andreas Popp, Morphosys AG
- Developmental challenges with recombinant antibody mixture
Christian Müller, Symphogen A/S
- Getting ready for commercial production
Harald Bradl, Boehringer Ingelheim
- Wrap up
Stephan Schlenker, Boehringer Ingelheim

LIEGE ROOM LEVEL 1

GE Healthcare

From 9h00 to 11h00

Implementation of novel upstream technologies – An integrated/systematic approach
This workshop will offer a unique insight into the impact of novel upstream strategies on bioprocess productivity, economics and reliability provided by globally recognized technologists and visionaries from the biopharmaceutical industry.
End User perspective:
Alain Pralong, GSK Biologics, Belgium, VP New Production Introduction and Industrialisation

- Industry Analyst perspective:
Miriam Monge, Biopharm Services Ltd, Vice President Sales & Marketing
- Contract Manufacturer Organization perspective:
Dethardt Müller, Rentschler Biotechnologie GmbH, Vice President Technology Development
- Technology Provider perspective and moderator:
Gerard Gach, GEHC Life Sciences, Cell Culture Strategy Director – Bioprocess
The Workshop brings together globally recognized technologists and visionaries from the biopharmaceutical industry. Through their in-depth experiences in implementing new processes, they have specific insights on the impact of upstream single use tools and strategies in the bioprocess environment. The audience will benefit from the panels knowledge on implementation and integration of new technologies and will be invited to join in the conversation. They will draw from studies of process modeling, supply chain concerns and CMO use of disposables in order to highlight the flexibility and safety benefits of single use in a full scale production environment. In addition, a preview of a next generation single use seed train bioreactor platform will be presented - the first time this instrument will be shown.

ARTOIS ROOM LEVEL 5

From 9h00 to 11h00

IrvineScientific

Establishing Scale-up Process Using a Platform Chemically Defined Medium in Single-Use Disposable Bioreactor Systems

Tom Fletcher

Case studies will be presented as examples of how innovation has helped overcome particular challenges during cell culture media development.

- Custom Serum-Free Media Development & Optimization for Stem Cells Expansion

Jessie H.-T. Ni, Ph.D. Chief Scientific Officer, Irvine Scientific

- Effects of Cell Culture Media on Therapeutic Protein Quality

Tom Fletcher, Director, Research and Development, Irvine Scientific

- Establishing a Scale-up Process for Single-Use Disposable Bioreactor Systems Using a Platform Chemically Defined Medium

Matt Caple, Scientific Director Cell Culture Development, Gallus BioPharmaceuticals, LLC

TURIN ROOM LEVEL 1

KERRY

From 11h15 to 13h15

Rapid, Cost Effective Supplementation Strategies to Optimize media for the production of rProteins, Biosimilars, and Vaccines while Minimizing Variability

John F. Menton, PhD - Cell Culture R&D Manager

Supplementation of cell culture media is recognized as perhaps the most effective means for delivering enhanced culture/process productivity while reducing development timelines. The task of screening, selecting, and characterizing the vast array of available media supplements can be daunting and costly. In this workshop, we will explain the steps taken by Kerry over the years to minimize variability in our products. We will also demystify and simplify much of the media optimization process, by presenting a technical overview on the most widely used supplements, common practices, recommended approaches to screening and selection, and how to avoid common mistakes with respect to supplementation strategies.

ARTOIS ROOM LEVEL 5

SAFC®

From 11h15 to 13h15

Better Risk Mitigation Strategies: Practical Regulatory and Safety Testing Advice for Biologics Manufacturers

Join us for a practical and interactive workshop discussing the latest in risk mitigation strategies, providing useful regulatory and safety testing advice, and reviewing advances in technologies for risk mitigation.

Presentations will be followed by an «ask the experts» session, so that you can pose your questions directly to our experienced panel, which includes:

- Kevin Kayser, Director, CHOZN® Product Development, SAFC

- Chas Hernandez, Senior. R&D Scientist, Virologist, SAFC

- Martin Wisher, Senior Director, Regulatory Affairs, SAFC

LIEGE ROOM LEVEL 1

sartorius stedim biotech

From 11h15 to 13h15

"Advances in single-use bioreactor technology"

Driven by a growing pipeline of biopharmaceutical drugs in development and the cost pressure that the Pharma industry is experiencing, more and more companies are adopting single-use bioreactors up to and beyond the 1000 L scale. Especially continuous processing has gained tremendous interest as it allows reducing production scale and facility footprint. Besides classical biopharmaceuticals and vaccines, cell therapy products are moving towards industrial relevance. All this increases technical, supply chain and quality requirements on single-use bioreactors significantly.

During the workshop we will discuss the current status of single-use bioreactor solutions covering scale-up, high cell density cultures, qualification and integrity testing.

- Implementation of single-use bioreactors for scale-up and biologics production

Dr. Anne Gilbert, Ph.D, USP Director, Product and Process Development, Novasep Process

- Key considerations for development and qualification of single-use bioreactors

Dr. Gerhard Greller, Director Upstream Technology, Sartorius Stedim Biotech

- Managing challenges of large scale, high cell density cultures; experiences from the XD® process

Dr. Gerben Zijlstra, DSM Biologics

- Converting cell therapy manufacture to pharmaceutical production – quantity Vs biology

Dr. Christian van den Bos, Lonza Collogne

- Integrity testing of single-use bioreactors and bags

Dr. Martin Dahlberg, Sartorius Stedim Biotech

EUROTOP AUDITORIUM LEVEL 5

Thermo SCIENTIFIC

From 9h00 to 13h15

1. Support your validation requirements

David Klinkenberg, BioProduction Scientist and Technical Advisor

The qualification and validation of production, harvest and containment supplies is an integral part of any biopharmaceutical process. Regulatory guidelines around the World recommend that the production, storage and packaging components be assessed for extractables or leachables that may interact with or impact the product being manufactured. Key topics covered:

- Source of extractable and leachable materials from polymeric containers
- How to satisfy requirements for regulatory compliance
- How to obtain the technical data you need from a supplier's documentation

2. 1 Media development and selection strategies that achieve optimal productivity – case studies detailing basal media and feed optimization and application of single-use technologies

Tariq Haq, Senior Product Manager, Media

Production clones have unique bioprocess requirements, making media and feed selection a challenge. As such, it is critical to identify optimal media and feeds, and then combine them with effective cell culture strategies to obtain peak productivity.

A proven method of media and feed optimization that has the ability to thoroughly evaluate nutrient demands of cell culture from high-throughput to production-scale is Metabolic Pathway Design™. Once a medium has been fully optimized through this process, designing the optimal feed strategy to sustain cell growth and generate greater expression in production-scale systems is necessary. Such optimization programs can notably impact productivity, significantly improve process improvement times, and reduce costs. Case studies will discuss successful application of Metabolic Pathway Design for basal media optimization, and feed optimization.

2.2. Development of a novel single-use technology for microcarrier cell culture harvests: Thermo Scientific HyQ Harvestainer

Don Young, Senior Product Manager, BPC

The launch of the Thermo Scientific Single-Use Bioreactor (SUB) in 2006 changed the way biopharmaceutical companies established biological manufacturing processes. Single-use technologies continue to replace stainless steel models – delivering significant advantages including design flexibility and cost-savings. Application-testing and continued integration of leading technologies ensure that single-use systems continue to drive performance in the bioprocessing industry.

A recent innovation, the HyQ Harvestainer, is a fully enclosed single-use solution for processing microcarrier beads in bioreactor cultures. For production processes using anchorage-dependent cells grown on microcarriers, the Harvestainer system provides the customer with a single-use bioprocess container for harvesting and separating the media and expressed biologic or vaccine from the microcarriers.

2.3 Critical process and supply chain services to improve bio-manufacturing performance

Garland Grant, Senior Product Manager, Collaborative Technologies

The bioprocessing industry has undergone rapid growth in the last decade. Technology, cell culture methods, regulatory demands and companies' focus continue to advance – creating new demands on how suppliers and the biologics manufacturers (bio-manufacturer) interact. Media optimization, feed strategy development, on-site process development support, analytical testing, and validation services are just a small handful of the collaborative technologies required to ensure optimal bio-manufacturing performance.

Successful supplier and bio-manufacturer relationships enable internal processes to be streamlined, leveraging the full strength of both organizations to achieve greater efficiency. This presentation will provide an overview of the services critical to achieving process efficiency gains and provide examples of how increased interaction through collaboration efforts enabled suppliers to deliver greater value to the bioprocessing industry.

EUROTOP AUDITORIUM LEVEL 5

THERAPEUTIC VACCINES

Tarit Mukhopadhyay, University College London, UK
Barry Buckland, University College London, UK
Topics to be covered:
Cell delivery of antigens (e.g. Dendritic Cells)
Virus delivery of antigens using a variety of host mammalian cells; for example adenovirus, Herpes, AAV
Development of appropriate safe human cell lines that can be grown in suspension culture
Lessons to be learned from traditional vaccines
Challenge of determining potency of the vaccine
Safety challenges; freedom from unwanted adventitious agents

- Development of a vaccine candidate for Leishmaniasis; a therapeutic vaccine in clinical trials
Paul Kaye, York University, UK
- Regulatory Approval of new cell lines
David Onions, previously CSO, Bioreliance
- Case Study: Retroviral Like particle hepatitis C vaccine
Manuel Carrondo, IBET
- "Plug-and-Play" platform for rapid, scalable, cost-effective manufacture of traditional and novel vaccines.
Peshwa Madhusudan, MAXCYTE, Exec VP Cellular Therapies

LIEGE ROOM LEVEL 1

METABOLISM AS A KEY FOR IMPROVEMENT OF CELL CULTURE PROCESSES - STATUS AND ADVANCES IN ANALYTICS AND DATA ANALYSIS

Yvonne Genzel, Max Planck Institute, Germany
Udo Reichl, Max Planck Institute, Germany
Monitoring of extracellular metabolites such as glucose, glutamine, lactate and ammonium is routinely performed in cell culture since many years. Together with data on cell concentration and viability the development of media, the establishment of basic mathematical models to characterize cell growth and product formation, and the optimization of production processes was supported. With the advent of systems biology, a multitude of new assays to describe basic cell properties have been implemented successfully. These include measurement of intracellular metabolites, nucleotides, proteins, lipids and enzyme activities. On the other hand, basic questions, for instance the impact of compartmentalization, the role of signal transduction processes on cellular metabolism, or the integration of data sets obtained with different tools and from several process stages remain to be addressed properly.

While it is clear that metabolism is a key to achieve robust cell growth and to maximize cell-specific product yields, collecting generic data alone does not enable further development of cell lines or optimization of cultivation processes. Unless the complex information obtained can be integrated into a coherent view on cell-specific properties relevant for production of biologicals, including quantitative models to analyze and to predict at least some aspects of cell behavior, the full potential of new assay technologies cannot be exploited.
Focusing on metabolism-related topics, the workshop intends to present an overview on the analytical portfolio of today and to discuss limitations and new insights from the generated data. The following questions will be addressed:
- What new assays have been developed over the recent years?
- What are the current limits in analyzing cellular metabolism?
- What options are available to design improved media?
- What solutions exist to cope with biological variance and batch-to-batch reproducibility to obtain statistical significant results?
- What is the status of mathematical modeling approaches?
- What would be required for a comprehensive description of relevant aspects of cell growth and product formation in manufacturing of biologicals?

The invited speakers will be
- Tools for metabolic characterization of animal cell processes
Ana Teixeira, IBET, Portugal
- How flexible are animal cells? What can we learn from intracellular metabolite and enzyme activity data?
Yvonne Genzel, Max Planck Institute, Germany
- Cellular compartmentalization: What are the consequences for metabolic studies with CHO cells?
Ralf Takors, University Stuttgart, Germany
- The effect of low nutrient concentrations on product quality during fed-batch cultures
Mike Butler, University Manitoba, Canada
- Options & challenges in modeling metabolism
Udo Reichl, Max Planck Institute, Germany

MATISSE ROOM LEVEL 8

THE WWW.CHOGENOME.ORG RESOURCE FOR THE INTERNATIONAL CHO BIOTECHNOLOGY COMMUNITY

Nicole Borth, Universität für Bodenkultur, Austria
Kelvin H. Lee, University of Delaware, USA
Mike Betenbaugh, Johns Hopkins University, USA

When the first CHO genomic sequence was published in 2011, the immediate need of the scientific community was to obtain easy and fast access to the sequence information. With the release of additional sequencing results also came the realization that additional and new tools are necessary to take full advantage of the available information. This has raised a series of questions: Has the initial goal of easy access been achieved? And should the focus of www.CHOgenome.org change? Should efforts expand to include additional data sets and tools? Should more sequencing data and other ´omics measurements be included?
In this workshop we would like to further the discussion on the next steps for the cell culture community surrounding the CHO Genome effort. Some potential topics include:

- How and for what purposes does the community use genome information?
- Where does the community perceive future requirements and developments?
- What other 'omics data sets and tools should be added?
- What are the future directions of the CHO genome community?

We will start with short presentations on current applications of genome scale information, where it is useful, and how has it helped in different research efforts (contributions are encouraged; please contact nicole.borth@boku.ac.at if you would like to present on two slides maximum) and then proceed to an open forum and discussion addressing questions of interest to the community.

VAN GOGH ROOM LEVEL 8

IS TOMORROW'S PROCESS FED-BATCH OR PERFUSION?

Véronique Chotteau, KTH, Sweden
Tim Charlebois, Pfizer, USA

Fed-batch operation has dominated the biopharmaceutical production horizon over the last several decades. The lower technical risk, high production yields and the importance of therapeutic antibodies and their production platforms have been important drivers of fed-batch supremacy. But is the tide turning in the direction of perfusion-based cell culture and continuous bioprocessing? Several combined factors have been pushing the biopharmaceutical industry to reconsider the long-term future of fed-batch technology: a desire to reduce bioreactor volumes to take advantage of disposable equipment and associated flexibility; the trend toward precision medicine and smaller, more patient-targeted, niche indication products; the availability of more robust perfusion devices; the potential ability of the technology to support consistent, high-quality, high-performance production of a broad range of biopharmaceutical molecules, including but not limited to mAbs; opportunity to leverage downstream processing efficiencies via generation of a more continuous feedstream; potentially favorable plant and process economics.
The workshop will present a review of the historical and factual reasons for perfusion's lower 'popularity', today's views of several industrial actors of fed-batch and perfusion technology and how they see the future of bioprocesses, as well as the comparative economic aspects comparing these modes of operation. This will be followed by a discussion with the workshop participants to explore what the future horizon might look like for the production of recombinant glycoproteins, antibodies, enzymes, viral vectors etc.

- Workshop introduction - Perfusion process perception today
Veronique Chotteau, KTH, Sweden
- Opportunities for driving enhanced performance of CHO-based bioprocesses
Greg Hiller, Pfizer, USA
- Fed-batch vs. perfusion for tomorrows biopharmaceutical
Jakob Rasmussen, NovoNordisk, Denmark
- Fed-batch and Perfusion Processes: Economic and Operational Considerations
Suzanne Farid, University College London, UK
- Fed-batch vs. perfusion for biopharmaceutical production
Bert Frolich, Shire, USA
- When and Why to use perfusion for high quality production of Viral Vectors and Vaccines
Amine Kamen, CNRC, Canada

SPEAKERS & CHAIRPERSONS INDEX

SUNDAY 23RD OF JUNE

VAUBAN AUDITORIUM LEVEL 3

16h00 **OPENING CEREMONY**
Yves-Jacques Schneider, 23rd ESACT Meeting Chair - Martin Fussenegger, ESACT Chairman

16h30 **KEYNOTE LECTURE:**
Adult Human Mesenchymal Stem Cells and their use in pre-clinical animal models of disease
Arnold Caplan, Case Western Reserve University, USA.

17h15 **SESSION I: CELL THERAPIES AND VACCINES**
Chairpersons: Paula Marques Alves & Spiros Agathos

17h15 Human pluripotent stem cells, a versatile tool full of promises for cell therapies
Marc Peschanski, INSERM Évry, France

17h50 Robust cell manufacturing platforms and novel proteomic approaches to streamline the design of cell-based therapies for myocardial infarction
Margarida Serra, IBET, Portugal

18h10 A novel genotype of mva that efficiently replicates in single cell suspensions
Ingo Jordan, ProBiogen, Germany

18h25 Need of rapid and universal quantification methods for influenza vaccine release
Amine Kamen, NRCMC, Canada

18h40 End of the SESSION I

19h00 Traders reception

LILLE GRAND PALAIS

at 19h00 in the EXHIBITION AREA

TRADERS COCKTAIL

VAUBAN AUDITORIUM LEVEL 3

9h00 **SESSION II: ADVANCED CELLULAR MODELS**
Chairpersons: Nicole Borth & Hansjörg Hauser

9h00 Novel hepatocyte cell lines with preserved primary-like phenotype
Christoph Lipps, HZI, Germany

9h20 Aspects of vascularization in multi-organ-chips
Reyk Horland, TU, Germany

9h40 Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo.
Ira Espuny-Camacho, University of Brussels, Belgium

10h00 Human neural in vitro models for preclinical research: 3d culture systems for differentiation and genetic modification of stem cells
Catarina Brito, IBET, Portugal

10h15 Improvement in a human ige-inducing system by in vitro immunization
Hiroharu Kawahara, Kitakyushu National College of Technology, Japan

10h30 Coffee Break in the Exhibition Area

11h00 Special Delivery: Targeted Gene Silencing
Judy Lieberman, Harvard Medical School, USA

11h35 Microrna biogenesis in CHO cells: the impact of dicer mediated Mirna processing on CHO cell phenotpye
Matthias Hackl, BOKU, Austria

11h55 Stable microrna expression improves antibody productivity in Chinese hamster ovary producer cells
Michaela Strotbek, University of Stuttgart, Germany

12h15 Novel strategy for a high-yielding mab-producing CHO strain (overexpression of non-coding rna enhanced proliferation and improved MAB yield)
Hisahiro Tabuchi, Chugai Pharmaceutical, Japan

JEANNE DE FLANDRES LEVEL 11

12h30-14h30 Lunch Break

12h30 Poster Session A in the Exhibition Area (even reference)

VAUBAN AUDITORIUM LEVEL 3

14h30 **SESSION III: EPIGENETICS AND SYNTHETIC BIOLOGY**
Chairpersons: Martin Fussenegger & Terry Papoutsakis

14h30 MicroRNA and omics
Kevin Lee, Delaware Biotechnology Institute, USA

15h05 Engineering of synthetic circuits for biomedical application
Haifeng Ye, ETH, Switzerland

15h25 Crosstalk of synthetic cassettes in defined chromosomal sites
Shawal Spencer, HZI, Germany

15h40 Programmable designer circuits performing biocomputing operations in mammalian cells
Simon Ausländer, ETH, Switzerland

15h50 A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells
Konrad Müller, University of Freiburg, Germany

16h15 First cpg island microarray for genome-wide analyses of DNA methylation in Chinese hamster ovary cells: new insights into the epigenetic answer to butyrate treatment
Anna Wippermann, Bielefield University, Germany

16h30 Coffee Break in the Exhibition Area

17h00 Gene regulation in chromatin: Insights from epigenomics and genome editing
Dirk Schübeler, Friedrich Miescher Institute for Biomedical Research, Switzerland

17h35 Targeting FcRn for therapy: from subcellular trafficking analyses to in vivo studies in mice
Sally Ward, UT Southwestern Medical Center, USA

18h40 End of the day

VAUBAN AUDITORIUM Level 3

9h00 **SESSION IV: GENETIC AND PROCESS ENGINEERING**
Chairpersons: Ashraf Amanullah & Stefanos Grammatikos

9h00 Designer nucleases - understanding the basics, improving their application
Toni Cathomen, University Medical Center, Germany

9h35 2D fluorescence spectroscopy for real-time aggregation monitoring in upstream processing
Karen Schwab, Institute of applied Biotechnology, Germany

9h55 Recombination-mediated cassette exchange (RMCE) for monoclonal antibody expression in a chok1-derived host cell line
Lin Zhang, Pfizer, USA

10h15 Implementation of a predictive screening strategy for cell cloning by automation and parallelization
Anke Mayer-Bartschmid, Bayer, Germany

10h30 Rapid construction of transgene-amplified CHO cell lines by cell cycle checkpoint engineering
Kyoungho Lee, University of Osaka, Japan

10h45 Engineering a mammalian cell line toolbox that exhibits multiple productivity and product quality profiles
Chapman Wright, Biogen, USA

11h05 Coffee Break in the Exhibition Area

11h30 Multi-gene engineering of mammalian cell metabolism: walking the steps towards hyper-productivity
Ana Filipa Rodrigues, IBET, Portugal

11h50 Monitoring intracellular redox changes in biotechnologically relevant mammalian cell lines with genetically encoded fluorescent biosensors
Karen Perelmuter, Institut Pasteur, Uruguay

12h10 A comprehensive view on an old metabolite: lactate
Martin Jordan, Merck Serono, Switzerland

12h25 Comprehensive understanding of heparan sulfate proteoglycan biosynthesis in CHO and hek293 cells
Sojeong Lee, Kaist, Korea

JEANNE DE FLANDRES Level 11

12h30-14h30 Lunch Break

12h40 Poster Session B in the Exhibition Area (odd references)

TURIN ROOM Level 1

12h40 - 14h30 **ESACT GENERAL ASSEMBLY**

14h30 End of lunch break

14h00 Outing - departure according to your TOUR (see page 6)

+ Dinner at the Museum of Fine Arts of Lille

19h30 – 21h00: Free exhibition visit of the Museum with conference guides.

21h00 – 23h00: Cocktail-Dinner

VAUBAN AUDITORIUM LEVEL 3

9h00 **SESSION V: NEXT GENERATION MOLECULE FORMATS**
Chairpersons: Francesc Godia & Hitto Kaufmann

9h00 Gene therapy: progress and challenges
Thierry Vanden Driessche, Free University of Brussels, Belgium

9h35 Targeting of siRNA to inflammatory diseases
Jorgen Kjems, Interdisciplinary Nanoscience Center iNANO, Denmark

10h10 Beat™ the bispecific challenge: a novel and efficient platform for the expression of bispecific IgGs
Pierre Moretti, Glenmark, Switzerland

10h25 Next generation bispecific antibody design and the influence thereof on product yield and stability
Karin Taylor, University of Queensland, Australia

10h45 Investigating the determinants of novel-format antibody expression in CHO cells
Claire Gaffney, University of Manchester, UK

11h00 A quantitative and mechanistic model for monoclonal antibody glycosylation as a function of nutrient availability during cell culture
Ioscani Jimenez Del Val, Imperial College, UK

11h15 Coffee Break in the Exhibition Area

11h30 **SESSION VI: CELL INTERACTIONS**
Chairpersons: Alan Dickson & Otto-Wilhelm Merten

11h30 From matrix mechanics to the nucleus
Dennis Discher, University of Pennsylvania, USA

12h05 Electrically modulated attachment and detachment of animal cells cultured on an ITO electrode.
Sumihiro Koyama, Jamstek, Japan

12h20 Announcements (ESACT courses, JAACT, ECI CCE etc…)

JEANNE DE FLANDRES LEVEL 11

12h30-14h30 Lunch Break

12h30 Poster Session C in the Exhibition Area

VAUBAN AUDITORIUM LEVEL 3

14h30 **KEYNOTE LECTURE:**

Engineering liver tissue from stem cells
Catherine Verfaillie, KU Leuven, Belgium

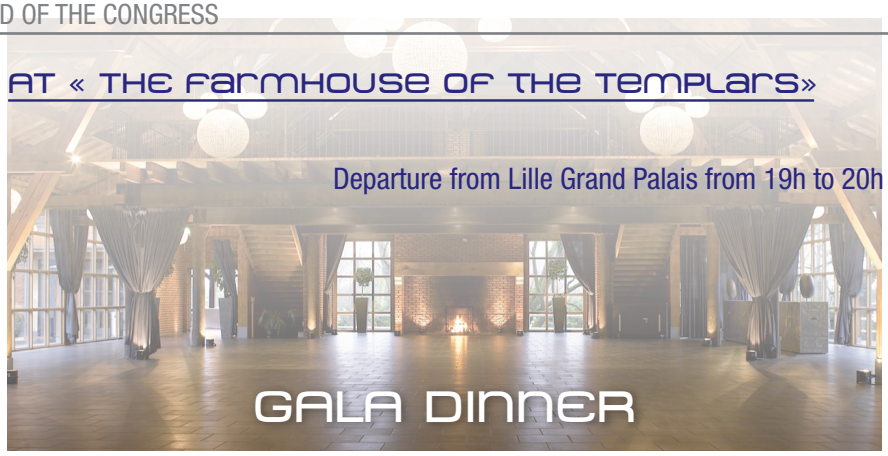
15h15 MERCK MILLIPORE POSTER PRIZE SESSION

17h15 CLOSING CEREMONY
Yves-Jacques Schneider - Francesc Godia - Hansjörg Hauser

17h30 END OF THE CONGRESS

AT « THE FARMHOUSE OF THE TEMPLARS»

Departure from Lille Grand Palais from 19h to 20h



ABSTRACTS SUMMARY

Sunday 23rd of June- 17h50
001

ROBUST CELL MANUFACTURING PLATFORMS AND NOVEL PROTEOMIC APPROACHES TO STREAMLINE THE DESIGN OF CELL-BASED THERAPIES FOR MYOCARDIAL INFARCTION

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KEY WORDS:
INDUCED PLURIPOTENT STEM CELLS / HUMAN CARDIAC STEM CELLS / ACUTE MYOCARDIAL INFARCTION / BIOREACTORS / HIGH-THROUGHPUT PROTEOMIC TOOLS

BACKGROUND AND NOVELTY:
Stem cell (SC) transplantation has emerged as an exciting treatment for patients with acute myocardial infarction (AMI). The major challenges in this field are lack of expertise in product characterization and specialized cell manufacturing which are imperative to bring SC-based products to clinic [1]. Within this context, our work has been focused on production and characterization of two challenging SC-based products: i) cardiomyocytes (CM) derived from induced pluripotent SCs (iPSC), which are capable to regenerate myocardium [2], and ii) adult cardiac SCs (hCSC), which trigger paracrine mechanisms that activate endogenous SCs to promote regeneration[3].

EXPERIMENTAL APPROACH:
Our strategy for CM production consisted in integrating cardiac differentiation and cell lineage purification steps in environmentally controlled stirred tank bioreactors. iPSC were cultivated as aggregates and the impact of different parameters on bioprocess yields was evaluated. Regarding hCSC expansion, different microcarriers were screened for their ability to support hCSC growth. Cell characterization was performed along culture time using flow cytometry, qRT-PCR and microscopy analysis. High-throughput proteomic tools have also been applied to uncover novel molecules of hCSC Receptome.

RESULTS AND DISCUSSION:
Our results showed that hypoxia conditions and an intermittent stirring profile are key parameters in iPSC differentiation towards functional CM. Using these conditions, we were able to improve by 1000-fold the final yields of CM (purity>98%).
An efficient protocol for hCSC cultivation using microcarrier technology was successfully implemented and hCSC retained their phenotype, multipotency and ability to secrete key growth factors after expansion in bioreactors. From hCSC Receptome analysis, more than 2000 proteins were identified, including proteins involved in the cardiac function.
The knowledge generated from our study will establish a new way to streamline the design and manufacturing of novel cell-based therapies for AMI.

Sunday 23rd of June- 18h10
002

A NOVEL GENOTYPE OF MVA THAT EFFICIENTLY REPLICATES IN SINGLE CELL SUSPENSIONS

Ingo JORDAN [1], Volker SANDIG [1]

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KEY WORDS:
VACCINES / MODIFIED VACCINIA ANKARA / AVIAN CELL LINE / AGE1.CR.PIX

BACKGROUND AND NOVELTY:
Vectored vaccines based on modified vaccinia Ankara (MVA, a hyperattenuated poxvirus) may lead to new treatment options against infectious diseases and certain cancers. We established avian suspension cell lines (CR and CR.pIX) and developed chemically defined media for production of MVA. Because cell-to-cell spread is an important mechanism for vaccinia virus replication one common hurdle in suspension processes appears to be that induction of cell aggregates is required to obtain high yields. We now describe a novel genotype of MVA that replicates to high titers in the CR single cell suspensions without aggregate induction.

EXPERIMENTAL APPROACH:
After passaging MVA in CR suspension cultures in chemically defined media we observed that infectious titers increased. 135 kb of genomic DNA sequence recovered from a passage eleven population revealed accumulation of a novel genotype (MVA-CR) with point mutations in three genes. Remaining traces of wildtype virus were removed by plaque purification and the pure isolate (MVA-CR19) was further characterized.

RESULTS AND DISCUSSION:
Compared to wildtype MVA, plaques formed by MVA-CR19 on adherent CR cells appear to be larger and to develop earlier. Titers are slightly higher in complete lysates and significantly elevated in cell-free supernatants. Most surprisingly, MVA-CR19 replicates efficiently without aggregate induction also in single cell suspension cultures. We hypothesize that a greater fraction of MVA-CR19 escapes the hosts to also infect distant targets. In such a model the new genotype should not confer a significant advantage to viruses spreading in cell monolayers, and indeed we could not generate the MVA-CR genotype by passaging in adherent cultures. Production and purification of MVA-based vaccines may be simplified with this strain as processes based on single cell suspensions are less complex compared to the current protocols and because infectious units accumulate in the cell-free volume where burden with host-cell derived contaminants is lower.

NEED OF RAPID AND UNIVERSAL QUANTIFICATION METHODS FOR INFLUENZA VACCINE RELEASE

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KEY WORDS:
INFLUENZA VACCINE / QUANTITATION / LOT RELEASE / PROCESS ANALYTICAL TECHNOLOGIES (PAT) / CELL CULTURE

BACKGROUND AND NOVELTY:
Hemagglutinin (HA), the major surface protein of the influenza virus, is used as the influenza vaccine potency marker and measured by Single Radial Immunodiffusion (SRID) assay. To date, SRID remains the only method approved by the regulatory agencies to release influenza vaccines. The method relies on the availability of reference reagents which need 2-3 months to be updated and produced. Clearly, novel rapid, robust and reliable in-process quantification methods to monitor viral particles and viral antigen content are highly needed to accelerate development and approval of candidate influenza vaccines within the timelines required by urgent interventions in case of pandemics.

EXPERIMENTAL APPROACH:
To respond to these urgent needs, many technologies are under development, but among different methods evaluated, a universal SRID assay using antibodies recognizing conserved HA regions and RP-HPLC method to quantify total HA content in vaccine doses were the most promissing. Whereas, anion-exchange HPLC to in-process monitor the total viral particles stands out as a valuable method to accelerate process development for influenza vaccine candidates.

RESULTS AND DISCUSSION:
The three methods have been successfully evaluated and compared to specific strain-SRID assay to quantify different HA strain subtypes in vaccine preparations. Furthermore, RP-HPLC and anion-exchange HPLC methods were successfully used to monitor HA antigen and total viral particles in samples at all stages of the manufacturing process. This set of universal, rapid and reliable methods is currently validated and is used to support an accelerated development of a cell culture process for influenza vaccine manufacturing. Also it is used to better define the quality attributes of the bulk product and accelerate the release of vaccine lots.

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Petiot E, Jacob D, Lanthier S, Lohr V, Ansorge S, Kamen AA: Metabolic and kinetic analyses of influenza production in perfusion HEK293 cell culture. BMC Biotechnol 2011, 11:84.

NOVEL HEPATOCYTE CELL LINES WITH PRESERVED PRIMARY-LIKE PHENOTYPE

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KEY WORDS:
HEPATOCYTES / IMMORTALIZATION / HEPATOCYTE LONG TERM CULTIVATION / ENGRAFTMENT / MAINTENANCE OF FUNCTIONALITY

BACKGROUND AND NOVELTY:
Hepatocytes (HCs) are highly specialized cells that display a number of specific/unique functions such as protein synthesis, carbohydrate metabolism and detoxification. These properties render hepatocyte cultures an attractive option for animal toxicology testing, screening for new drugs as well as regenerative approaches. However, such approaches are limited since primary HCs lose their specific features within few days of in vitro cultivation. Thus, they cannot be expanded to sufficient numbers. Since currently available cell lines are insufficiently expressing specific hepatic factors, we explored novel strategies for expansion of HCs by controlled immortalization. We show the generation of expandable HC cell lines which maintain many properties of freshly isolated hepatocytes and can even rescue animals that display compromised liver functions.

EXPERIMENTAL APPROACH:
A lentiviral screening library comprising more than 30 immortalizing genes was used to randomly infect primary mouse HCs of different genetic background. Cell cultures were screened for proliferation and characterized for the hepatic properties in 2D and 3D culture conditions.

RESULTS AND DISCUSSION:
Immortalized cell lines were established that showed robust proliferation and underwent more than 70 cumulative population doublings within the first 170 days. Combinations of genes were identified that reproducibly support expansion and high levels of hepatic markers including albumin, HNF4 , Foxa2 and C/EBP . Subjecting the cell lines to three-dimensional cultivation conditions, hepatic marker expression levels were found to increased and even closer to primary HCs. To evaluate if these cells can engraft and overtake functionality in vivo, they were transplanted into FAH-/- mice. While non-transplanted control animals died from liver failure, transplanted animals were rescued. Immunohistochemistry confirmed successful engraftment. Together, the results show that this strategy supports the expansion of HCs while preserving their specific functions.

Monday 24th of June- 9h20
005

ASPECTS OF VASCULARIZATION IN MULTI-ORGAN-CHIPS

Reyk HORLAND [1], Reyk HORLAND [1], Sven BRINCKER [1], Benjamin GROTH [1], Ulrike MENZEL [1], Ilka WAGNER [1], Eva-Maria MATERNE [1], Gerd LINDNER [1], Alexandra LORENZ [1], Silke HOFFMANN [1], Mathias BUSEK [1], Frank SONNTAG [3], Udo KLOTZBACH [3], Roland LAUSTER [1], Uwe MARX [1, 2]

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KEY WORDS:
MULTI-ORGAN-CHIP / MICRO-BIOREACTOR / ENDOTHELIAL CELLS / ARTIFICIAL VASCULATURE / CIRCULATION SYSTEM

BACKGROUND AND NOVELTY:
Enormous efforts have been made to develop circulation systems for physiological nutrient supply and waste removal of in vitro cultured tissues. However, none of the currently available systems ensures long-term homeostasis of the respective tissues over months. This is caused by a lack of in vivo-like vasculature, which leads to continuous accumulation of protein sediments and cell debris in the systems. Here, we demonstrate a closed and self-contained circulation system emulating the natural blood perfusion environment of vertebrates at tissue level.

EXPERIMENTAL APPROACH:
The system uses a miniaturized physiological blood-like circulatory network with an integrated micro-pump to provide circulation of microliter-volume to support milligrams of tissue. This mimics the physiological ratio of humans, where liters of blood-volume support kilograms of tissue, at a chip-compatible micro-scale. The self-contained circulation systems were formed in PDMS by replica molding from master molds and were afterwards bonded to a cover-slip by oxygen plasma treatment. Human microvascular endothelial cells (HMVEC) were seeded into the channels, attached to all channel surfaces, and afterwards cultured up to 14 days under pulsatile flow conditions.

RESULTS AND DISCUSSION:
We evaluate the impact of artificial vessels in an approach for systemic substance testing in multi-organ-chips. Creating the conditions for the circulation of nutrients through the tissue-chamber, will allow for in vivo-like crosstalk between endothelial cells and tissues and prevent clumping inside the channels. Data on the colonization of the microfluidic 3D channels and long-term viability of the endothelial cell layers will be presented. In addition, challenges and opportunities of this platform technology in comparison to the existing dynamic bioreactor systems will be addressed.

Monday 24th of June- 9h40
006

PYRAMIDAL NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS INTEGRATE EFFICIENTLY INTO MOUSE BRAIN CIRCUITS IN VIVO

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KEY WORDS:
HUMAN PLURIPOTENT STEM CELLS / CORTICAL DIFFERENTIATION / TEMPORAL PATTERNING / IN VIVO TRANSPLANTATION AND INTEGRATION INTO THE HOST / AXOGENESIS AND NEURONAL MATURATION

BACKGROUND AND NOVELTY:
The cerebral cortex is the most complex structure of our brain. During evolution, the relative size of the cortex has increased considerably among higher mammals and new cortical areas involved in higher evolved functions have emerged. The study of human cortical development has major implications for brain evolution and cortical related diseases, but has remained elusive due to paucity of experimental models.
Here, we describe an intrinsic pathway of corticogenesis from human embryonic (ESC) and induced pluripotent (iPSC) stem cells leading to the sequential generation of first forebrain progenitors and later pyramidal neurons of all six layers identities in a time-dependent fashion, highly reminiscent of the in vivo situation.

EXPERIMENTAL APPROACH:
We describe an in vitro model for the directed differentiation of human pluripotent stem cells in a monolayer fashion and devoided of morphogens, but supplemented with noggin, and inhibitor of the BMP pathway, that has been shown to be required for neuroectoderm specification. Specified progenitors and neurons are later transplanted into mouse newborn brain and analysed after several months in vivo by immunofluorescence analysis and by patch-clamp recordings.

RESULTS AND DISCUSSION:
Following this in vitro differentiation human pluripotent stem cells efficiently differentiated into forebrain and telencephalic progenitors based on the expression of various genes tested by immunofluorescence, quantitative PCR and microarray analysis. At later stages, these cells exited cell cycle and became cortical pyramidal neurons, as attested by their pyramidal morphology, but also by the expression of various markers of cortical neurons and cortical layer specific genes. The cortical neurons present markers of connectivity and a mature electrophisiological profile at later stages. Moreover, following grafting into the mouse newborn cortex, the human ESC-derived neurons extend axons to endogenous cortical targets, present numerous synapses and functionally integrated into the host.

HUMAN NEURAL IN VITRO MODELS FOR PRECLINICAL RESEARCH: 3D CULTURE SYSTEMS FOR DIFFERENTIATION AND GENETIC MODIFICATION OF STEM CELLS

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KEY WORDS:
I3D CELL MODELS / NEURAL DIFFERENTIATION / DYNAMIC CULTURE SYSTEMS / HUMAN STEM CELLS / GENE DELIVERY

BACKGROUND AND NOVELTY:
There is an increasing need for more relevant human cell models for the early stages of drug development. These models should closely recapitulate the in vivo cell-cell interactions and present higher physiological relevance. Here we describe the development of human 3D neural in vitro models for target validation and toxicological assessment, by combining human stem cells (SCs) as scalable supply of neural-subtype cells and dynamic culture systems.

EXPERIMENTAL APPROACH:
Dynamic 3D culture system-based strategies were adopted for expansion and differentiation of human SCs, namely NT2 embryocarcinoma SC line and midbrain-derived neural SCs (hNSC). Process parameter optimization included stirring rate, process duration, media composition and oxygen levels. Furthermore, in order to increase the versatility of the 3D models, we assessed the possibility of genetic manipulation strategies via helper-dependent canine adenovirus type 2 (hd-CAV2) vectors. Differentiation and transduction efficiencies were analyzed by phenotypic and functional characterization using confocal microscopy, electron microscopy, qRT-PCR, Western Blot and metabolic profiling.

RESULTS AND DISCUSSION:
NT2 differentiation in stirred tank vessels resulted in 3D co-cultures of neurons capable of synaptic activity and mature astrocytes, with 10 and 2.5 fold higher cell yields, respectively, in comparison with 2D cultures. Moreover, this process was reproducible and robust enabling an efficient cell source to feed high throughput toxicological assays. Concerning hNSC, differentiated neurospheres were enriched in neurons which expressed the dopaminergic markers tyrosine hydroxylase (TH) and Nurr1. Furthermore, hd-CAV2-mediated gene delivery strategies allowed an efficient gene transfer into differentiated neurospheres, with transgene expression detectable also in the inner layers.
The 3D models developed herein contribute to increase the collection of tools available for more accurate pre-clinical evaluation, accelerating the drug development pipeline.

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The authors acknowledge Dr J Schwarz and Dr S. Schwarz (U. Leipzig, Germany) for the supply of hNSC utilized within the scope of the EU project BrainCAV (FP7-222992); this work was also supported by PTDC/EBB-BIO/112786/2009 and PTDC/EBB-BIO/119243/2010, FCT, Portugal; BrainCAV (FP7-222992), EU.

IMPROVEMENT IN A HUMAN IGE-INDUCING SYSTEM BY IN VITRO IMMUNIZATION

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KEY WORDS:
IGE INDUCTION / ALLERGY / IN VITRO IMMUNIZATION / HUMAN LYMPHOCYTES

BACKGROUND AND NOVELTY:
The immune system, which is the self-defense system of the body, occasionally responds in a manner that is harmful to the body. The incidence and severity of allergies caused by the environment are increasing and have recently become a serious social problem. We have previously developed an in vitro system for inducing human IgE antibody specific to a designated antigen that can be used to study various allergic reactions.

In this study, we attempted to improve this system to stimulate IgE levels in its medium for a highly sensitive screening.

EXPERIMENTAL APPROACH:
The in vitro IgE-inducing system contained E-RDF supplemented with human plasma (final concentration, 10%); FCS (5%); IL-2, IL-4, and IL-6 (10 ng/ml each); and MDP (10 µg/ml), as described previously. Human lymphocytes were cultured in 96- or 24-well plates at a final density of one million cells/ml in the medium and incubated in a CO2 incubator at 37°C for 10 days. After 10 days, approximately 700 ng/ml of IgE antibody was secreted into the medium.

RESULTS AND DISCUSSION:
The IgE-inducing system was used to investigate various factors stimulating IgE production. When human lymphocytes obtained from naturally immunized donors with allergens were used, elimination of IL-2 from the medium gave higher IgE production. These results indicate that IL-2 may be required to initially immunize humans with allergens.
The level of secreted IgE reported in this study may be the highest compared to those reported elsewhere. This improved system for human IgE production in a medium without IL-2 is considered to be of profound use for studying allergy mechanisms and investigating allergy-alleviating materials.

Monday 24th of June- 11h35
009

MICRORNA BIOGENESIS IN CHO CELLS: THE IMPACT OF DICER MEDIATED MIRNA PROCESSING ON CHO CELL PHENOTPYE

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KEY WORDS:
CHO / MICRORNA / CELL ENGINEERING / GROWTH

BACKGROUND AND NOVELTY:
MicroRNAs are 19-24 nt long RNAs that control gene expression by translational repression or mRNA degradation. Applications of miRNAs in cell culture technology are the use as biomarkers during cell line development as well as gene engineering targets to enhance space-time yields of bioprocesses. The analysis of miRNA expression in CHO cells in response to serum supplementation showed a predominant up-regulation, which was reflected in higher expression of Dicer, the key enzyme in the production of mature miRNA duplexes. Since serum significantly enhances growth of CHO cells, we set out to study the relevance of Dicer for CHO cell growth.

EXPERIMENTAL APPROACH:
High-throughput analysis of miRNA transcription was done using Illumina and microarrays. MiRNA content was measured by chip-based gel electrophoresis. Dicer expression was analyzed by real-time PCR and Western Blot, and expression was manipulated using specific shRNA constructs and human Dicer cDNA expression constructs.

RESULTS AND DISCUSSION:
Based on the effect of serum supplementation on miRNA levels, Dicer expression was analyzed in response to growth arrest by serum-removal or nutrient depletion, which showed a strong down-regulation under these conditions. To further assess the link between CHO cell growth and Dicer expression, mRNA and protein levels were determined in five serum-free adapted CHO cell lines with specific growth rates ranging between 0.45 and 1.00 d-1. This data showed a strong correlation between Dicer expression and specific growth rate. A CHO DUKX-B11 host cell line was transfected with a human Dicer1 cDNA construct to generate two stable Dicer overexpressing host cell lines exhibiting a 1.5 – 2.5 fold overexpression. Compared to the untransfected host, a ~20-30% increase in growth rate and cumulative viable cell days was observed.
These data suggest that Dicer expression and hence the production of mature miRNAs is strongly linked to cell specific growth rate and stress response to nutrient limitation in CHO cells.

Monday 24th of June- 11h55
010

STABLE MICRORNA EXPRESSION IMPROVES ANTIBODY PRODUCTIVITY IN CHINESE HAMSTER OVARY PRODUCER CELLS

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KEY WORDS:
MICRORNA SCREEN / THERAPEUTIC PROTEINS / CELL LINE ENGINEERING / FED-BATCH

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate the expression of different target genes and, thus, potentially offer the opportunity to engineer networks of genes in order to achieve complex phenotypic changes in mammalian cells. We hypothesized that this feature of miRNAs could be exploited as a strategy to improve therapeutic protein production processes by increasing viable cell densities and/or productivity of mammalian host cells. This first functional miRNA screen in Chinese hamster ovary (CHO) producer cells led to the identification of miRNAs that enhance IgG productivities not only transiently but also in fed-batch cultures using stable cell lines.

EXPERIMENTAL APPROACH:
To identify miRNAs that increase the productivity of producer cells, a global miRNA screen was performed in CHO cells stably expressing an IgG1. In the primary screen, antibody titers in cell culture supernatants were determined upon transient transfection of a human miRNA library. Candidate miRNAs were validated in a secondary screen in terms of specific productivity in three different CHO producer cell lines. Finally, stable miRNA-expressing CHO producer cells were generated and their performance was analyzed in fed-batch cultures.

RESULTS AND DISCUSSION:
Our global screen identified 16 human miRNAs that reproducibly improved IgG titers when transiently introduced into CHO producer cell lines. Two miRNAs positively impacting the viable cell density and specific productivity, respectively, were selected and stably co-expressed in CHO producer cells. Preliminary results with these cells revealed higher IgG titers in fed-batch cultures while conserving product quality. MiRNA-based cell line engineering is thus an attractive approach toward the genetic optimization of CHO host cells for industrial applications.

NOVEL STRATEGY FOR A HIGH-YIELDING MAB-PRODUCING CHO STRAIN (OVEREXPRESSION OF NON-CODING RNA ENHANCED PROLIFERATION AND IMPROVED MAB YIELD)

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KEY WORDS:
LONG NON-CODING RNA / OVEREXPRESSION / HIGH-TITER / NFKBIA / COMPLEMENTARY SEQUENCE

BACKGROUND AND NOVELTY:
Innovation in mAb production is driven by strategies to increase yield. A host cell line constructed to overexpress TAUT (taurine transporter) produced a higher proportion of high-mAb-titer strains. From these we selected a single TAUT/mAb strain that remained viable for as long as 1 month. Its improved viability is attributed to improved metabolic properties. It was also more productive (>100 pg/cell/day) and yielded more mAb (up to 8.1 g/L/31 days) than the parent cell line. These results suggested that this host cell engineering strategy has great potential for the improvement of mAb-producing CHO cells.

EXPERIMENTAL APPROACH:
Our present challenge was to achieve a high yield in a shorter culture period by modulating events in the nucleus by using non-coding RNA (ncRNA). We looked for long ncRNA (lncRNA) that was abnormally expressed in high-titer cells. A Mouse Genome 430 2.0 array (Affymetrix) identified the lncRNA as a complementary sequence of the 3' non-coding region of mouse NFKBIA (NF-kappa-B inhibitor alpha) mRNA. NFKBIA is an important regulator of the transcription factor NFKB, a positive regulator of cell growth. Since NFKBIA suppresses NFKB function, inhibition of NFKBIA by overexpression of the lncRNA might further enhance cell proliferation. We genetically modified the TAUT/mAb strain to overexpress part of the lncRNA.

RESULTS AND DISCUSSION:
The resulting co-overexpression strains gave increased yield, and one strain increased yield in a shorter culture period (up to 6.0 g/L/14 days from 3.9 g/L/14 days). Interestingly, however, this effect might not be due to enhancement of the NFKB-dependent promoter activity of the mAb expression plasmid because mAb production under EF-1 promoter without an NFKB binding site was also enhanced by overexpression of part of the lncRNA. Since overexpression of the partial sequence still functions as an antibody production enhancing sequence in mAb-producing cell lines, many unexpected functions from ncRNA-containing microRNA might exist.

ENGINEERING OF SYNTHETIC CIRCUITS FOR BIOMEDICAL APPLICATIONS

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KEY WORDS:
SYNTHETIC BIOLOGY / SYNTHETIC GENE CIRCUITS / PROSTHETIC NETWORKS / GENE REGULATION / CELL THERAPY

BACKGROUND AND NOVELTY:
Synthetic biology has significantly advanced the design of genetic devices that can reprogram metabolic activities in mammalian cells and provide novel therapeutic strategies for gene- and cell-based therapies. In this study we designed a novel synthetic optogenetic transcription device used for the treatment of diabetes. Further more, we have assembled a designer circuit which allowed a three-in-one treatment strategy to simultaneously address metabolic syndrome: hypertension, hyperglycemia, and obesity.

EXPERIMENTAL APPROACH:
A synthetic signaling network enabling light-inducible transgene expression in mammalian cells was designed by linking the signal transduction of melanopsin to the control circuit of the nuclear factor of activated T cells. The engineered cells containing the designed circuit were subcutaneously or transdermally implanted into mice to remote control glucacon-like peptide 1 expression through illumination. Another assembled synthetic circuit was also designed which allowed an antihypertensive drug to dose-dependently regulate a chimeric trace amine-associated receptor whose singling was rewired to expression of the bifunctional peptide GLP-1-Leptin. This synthetic circuit was engineered into mammalian cells and further implanted into mouse models for disease therapy.

RESULTS AND DISCUSSION:
The optogenetic transcription device could be used to precisely program gene expression in mammalian cells and mice when illuminated by light. In type 2 diabetic mice subcutaneously implanted with the engineered light-responsive cells, the light-triggered expression of GLP-1 could successfully attenuate glycaemic excursions in db/db mice. The designer circuit was successfully tested in a mouse model of the metabolic syndrome. Mice containing engineered cell implants treated with guanabenz showed decreased blood pressure and significantly increased GLP-1 and Leptin levels which in turn attenuated glycemic excursions, decreased food intake, body weight, plasma cholesterol and free fatty acid levels.

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We thank Ignacio Provencio for providing pIRES2-OPN4AI, Hiroaki Matsunami for providing Hana3A cells, and Henryk Zulewski for critical comments on the work. This work was supported by the Swiss National Science Foundation (grant no. 31003A-126022), the European Research Council advanced grant and in part by the European Commission Seventh Framework Programme (Persist).

Monday 24th of June- 15h25
013

CROSSTALK OF SYNTHETIC CASSETTES IN DEFINED CHROMOSOMAL SITES

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KEY WORDS:
PROMOTER CROSSTALK / RMCE / ROSA26/TET-PROMOTER INTERACTION / EPIGENETIC SILENCING / DNA METHYLATION

BACKGROUND AND NOVELTY:
Development of recombinant cell lines relies mostly on illegitimate recombination followed by extensive screening to select the best clones. This is required since chromosomal elements next to the transgene integration sites affect its expression ("position effect"). To investigate the expression of the Dox-inducible Tet-promoter within the well-known Rosa26 locus, we employed recombinase mediated cassette exchange. Unexpectedly, we saw stochastic transgene expression. We show that the Tet-promoter is prone to DNA methylation in this integration site suggesting a crosstalk between the incoming synthetic promoter and the endogenous Rosa26 locus.

EXPERIMENTAL APPROACH:
Site specific transgene integration in ES cells and NIH3T3 cells was pursued by Flp mediated RMCE. Transgene expression was quantified using FACs analysis, Luciferase assay as well as RT PCR. To investigate the epigenetic crosstalk DNA methylation was studied using bisulfite sequencing.

RESULTS AND DISCUSSION:
Various Tet-cassettes were targeted into the Rosa 26 promoter by employing Flp mediated Recombinase mediated cassette exchange (RMCE) technology. Depending on the design, the Tet-cassettes were found to be insufficiently expressed in various cell lines but also in transgenic mice. Unexpectedly, we observed only stochastic transgene activation within individual cells of the genetically identical clones. Epigenetic characterization revealed that the Tet promoter is highly affected by DNA methylation in the Rosa 26 locus in spite of the Rosa locus staying largely unmethylated itself. This work increases our understanding of the transgene behaviour upon targeting in various different loci and thus holds significant importance in establishment of transgenic cell lines and animal models.

Monday 24th of June- 15h37
014

PROGRAMMABLE DESIGNER CIRCUITS PERFORMING BIOCOMPUTING OPERATIONS IN MAMMALIAN CELLS

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KEY WORDS:
SYNTHETIC BIOLOGY / BIOCOMPUTING / BIOENGINEERING / GENE NETWORKS

BACKGROUND AND NOVELTY:
Cells operate as information-processing systems that dynamically integrate and respond to environmental input signals. In Synthetic Biology, bioengineers hijack existing or create novel gene circuits to perform tailored functions in living cells. In this study, existing biological parts are rewired to multi-component designer circuits operating in single mammalian cells. Programmed by external input signals, circuit-transgenic cells are capable of executing a set of basic Boolean logic gates as well as basic arithmetic operations that are reminiscent to digital circuits in electronics.

EXPERIMENTAL APPROACH:
The design strategy includes small molecule-dependent transcriptional regulators that integrate input signals and drive the transcription of downstream translational controllers or reporter genes. Functional interconnection of orthogonal gene controllers enables precise regulation of reporter protein production based on combinatorial control of transcriptional and translational gene switches. Fluorescent reporter proteins served as output signals and enabled single-cell analysis using fluorescence microscopy and flow cytometry.

RESULTS AND DISCUSSION:
Inspired by digital electronics, we first developed a set of cellular Boolean logic gates that are programmed by external inputs. Further connection of basic logic gates remarkably increased the computational capacity in single cells, which is exemplified by the design of the XOR gate. Additionally to logic gates, we developed two arithmetic circuits, the half-adder and half-subtractor, which both performed binary calculations in living cells. Biocomputing cells that are capable of executing basic arithmetic operations represent fundamental building blocks for future (multi-)cellular systems executing functions with increasing complexity. Linking biocomputing circuits to the detection of disease-related biomarkers could increase the precision of prosthetic gene networks and may pave the way for a new generation of cell-based treatment strategies.

A RED/FAR-RED LIGHT-RESPONSIVE BI-STABLE TOGGLE SWITCH TO CONTROL GENE EXPRESSION IN MAMMALIAN CELLS

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KEY WORDS:
OPTOGENETICS / GENE EXPRESSION / PHYTOCHROME / SYNTHETIC BIOLOGY / LIGHT-INDUCED

BACKGROUND AND NOVELTY:
Processes in multicellular systems are orchestrated by gene expression programs that are tightly regulated in time and space. The targeted manipulation of such processes by synthetic tools with high spatiotemporal resolution could, therefore, open new opportunities in tissue engineering and enable a deepened understanding of developmental processes. Here, we describe the first red/far-red light-triggered gene switch for mammalian cells for achieving gene expression control in time and space.

EXPERIMENTAL APPROACH:
We constructed the red light-switchable gene expression system based on the concept of a split transcription factor. In doing so, we capitalized on the red light-dependent interaction of the A. thaliana proteins phytochrome B (PhyB) and the phytochrome interacting factor 6 (PIF6). We optimized and characterized the system using secreted alkaline phosphatase (SEAP) as reporter and quantitatively analyzed the light-induced expression kinetics by a mathematical model. Finally, we used an in-vivo angiogenesis assay as a proof-of-concept for the system's suitability for applications in tissue engineering.

RESULTS AND DISCUSSION:
We show that the system can be toggled between stable on- and off-states using short light pulses at 660 or 740 nm. Gene expression correlated with the applied photon number and was compatible with different cell lines, including human primary cells. Experimental data and modeling results confirmed that expression shut-off occurs immediately upon illumination with far-red light. We demonstrate the system's potential in tissue engineering by applying it to induce angiogenesis in chicken embryos. The system's performance combined with tissue-compatible regulating red light will enable unprecedented spatiotemporally controlled molecular interventions in cells, tissues and organisms. Moreover, non-invasive induction by light represents an excellent alternative to existing processes for the production of biopharmaceuticals that are unstable or have cytotoxic or cytostatic properties.

FIRST CPG ISLAND MICROARRAY FOR GENOME-WIDE ANALYSES OF DNA METHYLATION IN CHINESE HAMSTER OVARY CELLS: NEW INSIGHTS INTO THE EPIGENETIC ANSWER TO BUTYRATE TREATMENT

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KEY WORDS:
CHINESE HAMSTER OVARY (CHO) CELLS / EPIGENETICS / DNA METHYLATION / CPG ISLAND MICROARRAY / BUTYRATE EFFECT

BACKGROUND AND NOVELTY:
Today a majority of biopharmaceuticals is produced in CHO cells. Optimisation of productivity and growth requires insight into regulatory processes which is to some extent accomplished by 'omics' approaches. A promising aspect in this context is the epigenetic process of DNA methylation. Supplementation of butyrate provides an opportunity to enhance cell specific productivities in CHO cells and leads to alterations of epigenetic silencing events. Genome-wide studies of changes in DNA methylation following butyrate treatment promise valuable information regarding the optimisation of cultivation processes. However, DNA methylation has not been investigated on a genomic scale in CHO cells so far, and suitable tools did not exist until recently. Here, we present a customised microarray allowing us to conduct genome-wide analyses of DNA methylation in CHO cells.

EXPERIMENTAL APPROACH:
In order to screen for differential methylation of genomic regions prone to encounter epigenetic modifications, so-called CpG islands (CGIs), we developed a 60 K microarray covering 19,598 promoter-associated and intragenic CGIs. The design was based on the genomic and transcriptomic information currently available for CHO cells. We analysed four replicate CHO DP-12 cultures prior to treatment with butyrate as well as 24 h and 48 h after butyrate addition.

RESULTS AND DISCUSSION:
We found 1,340 genes to be differentially methylated 24 hours after butyrate addition. GO terms regarding apoptosis, chromatin modification and DNA repair were significantly enriched. Functional classifications indicated involvement of several major signalling systems such as the Wnt and MAPK pathways. Moreover, the results hint towards a role of the miRNA system in the epigenetic answer to butyrate treatment. Genes of the major mediators of maintenance and de novo methylation, DNMT3A and UHRF1, showed differential methylation. Surprisingly, the observed regulations proved to be temporary, as 91 % of them were not detectable anymore another 24 hours later.

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Tuesday 25th of June- 9h35
O17

2D FLUORESCENCE SPECTROSCOPY FOR REAL-TIME AGGREGATION MONITORING IN UPSTREAM PROCESSING

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KEY WORDS:
SYNCHRONOUS FLUORESCENCE SPECTROSCOPY / EXTRINSIC FLUORESCENCE DYES / PROTEIN AGGREGATION / PROCESS CONTROL

BACKGROUND AND NOVELTY:
Product aggregation is one side effect of rising yields due to process improvement and therefore accompanied with massive product loss during downstream processing. Hence, real time bioprocess monitoring and on-line product quality control during upstream processing (USP) comes more into focus, addressing this issue. For bioprocess control, synchronous fluorescence spectroscopy (SFS) in combination with multivariate data analysis (MVA) based on intrinsic cell culture fluorescence is a promising tool. Furthermore extrinsic fluorescence dyes are widely used to detect and quantify aggregated protein. In this study, SFS in combination with extrinsic fluorescence dyes for further process optimization was investigated, in order to establish real-time aggregation monitoring in USP.

EXPERIMENTAL APPROACH:
The sensitivity of the extrinsic dyes and their detection limit regarding aggregated protein was estimated based on SFS and MVA for cell free model systems and in cell culture experiments. SEC-MALS and Protein A-HPLC were used as reference methods for determination of protein concentration, aggregation levels and molecular weight estimation. Furthermore, a CHO cell line producing a monoclonal antibody was used for cell culture experiments. The toxicity and biocompatibility of different fluorescence dyes were compared based on LC50 via WST1 assays and flow cytometry.

RESULTS AND DISCUSSION:
The detection limit within cell free model systems for protein aggregation monitored via extrinsic fluorescence dyes and SFS was strongly depending on the dye. As expected, fluorescence signal intensities and dye concentrations were correlated and the biocompatibility of extrinsic dyes varied. Suitable candidates were selected based on their sensitivity and toxicity. Additionally, dye accumulations within cells or onto membranes were identified and possible side effects towards aggregation real-time monitoring were analyzed. Cell culture experiments with extrinsic dyes gave additionally promising results towards detection limits.

Tuesday 25th of June- 9h55
O18

RECOMBINATION-MEDIATED CASSETTE EXCHANGE (RMCE) FOR MONOCLONAL ANTIBODY EXPRESSION IN A CHOK1-DERIVED HOST CELL LINE

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KEY WORDS:
MAB / RECOMBINATION-MEDIATED CASSETTE EXCHANGE / SITE-SPECIFIC INTEGRATION / FRT FLP / CELL LINE DEVELOPMENT

BACKGROUND AND NOVELTY:
Cell lines suitable for therapeutic monoclonal antibody (mAb) production require excellent growth, stability and productivity characteristics. The development of such cell lines has classically been a time-consuming and resource-intensive process. The objective of current study is to develop a FRT-/FLP based site-specific integration (SSI) to efficiently target gene of interest to a specific locus in CHOK1 genome and rapidly generate stable cell lines with desired performance characteristics. The presentation will demonstrate with examples how SSI has been successfully applied to the generation of high-performing mAb expressing cell lines. To our knowledge this is the first successful demonstration of FLP-based site-specific targeted mAb production in a widely used commercially relevant cell line.

EXPERIMENTAL APPROACH:
We engineered FRT sequences into a mAb expression vector and then performed a standard cell line construction process in a CHOK1-derived host cell line. High performance recombinant cell lines, harboring the modified mAb expression vector containing FRT sequences were isolated. One particular clone with the best combination of growth, productivity and stability characteristics combined with a low copy number and single integration site was used as a progenitor for the creation of the SSI host cell line by RMCE with a null vector. RMCE reaction replaces the original mAb transcription units in the progenitor cell line with marker genes to generate the new host cell line. This allows a more regulatory favorable RMCE, as the SSI host cell line is free of pre-existing mAb gene.

RESULTS AND DISCUSSION:
The resulting SSI recombinant cell lines not only exhibited excellent and consistent growth/productivity profiles, but importantly inherited the production stability trait observed in the progenitor. We have subsequently determined the genetic context of FRT-tagged loci in the SSI host by using Illumina HiSeq sequencing technology.The locus has been mapped to the reference CHOK1 genome published by BGI.

IMPLEMENTATION OF A PREDICTIVE SCREENING STRATEGY FOR CELL CLONING BY AUTOMATION AND PARALLELIZATION

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KEY WORDS:
CELL LINE DEVELOPMENT / AUTOMATION / PREDICTIVE CLONE SELECTION / SHORTEN DEVELOPMENT TIMELINES

BACKGROUND AND NOVELTY:
Despite many advances and novel techniques, cell line development is often rate-limiting in the overall progress of a specific project. To save on overall project timelines, it is Bayer's approach to push up cell line development into late research phase to have a high performing cell line ready before entering into clinical development. This comes at the expense of having to perform cell line development for multiple clinical candidates for a given project at the same time, with project load ever increasing as often observed in industry. To overcome these limitations the workflow has been optimized including a compact flexible automation platform as key component with integrated data management connected to our Biologics data platform.

EXPERIMENTAL APPROACH:
A new automation friendly cell line development workflow was created aiming at enhanced predictivity for scale-up while enabling automate screening of high numbers of different clones. Key steps were tested manually. The newly designed workflow is being transferred onto a specifically designed automation platform.

RESULTS AND DISCUSSION:
The newly designed workflow was manually tested and yielded clones of good productivity. An automation platform consisting of different liquid handlers, a cell imaging system, an incubator, a cryo vial decapper and a robo arm was established. First test runs were successfully started. The automation enables an efficient high throughput cell line development process. Appropriate data management is employed to cope with the associated increase in complexity of several candidates/projectes in parallel screening programs. A stringent workflow has been devised which is amenable to automation and still compatible with predictive clone selection and early assessment of product quality attributes.

RAPID CONSTRUCTION OF TRANSGENE-AMPLIFIED CHO CELL LINES BY CELL CYCLE CHECKPOINT ENGINEERING

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KEY WORDS:
CHINESE HAMSTER OVARY CELLS / GENE AMPLIFICATION / MONOCLONAL ANTIBODY / ATR / CELL CYCLE CHECKPOINT

BACKGROUND AND NOVELTY:
The process of establishing high-producing cell lines for the manufacture of therapeutic proteins is both time-consuming and laborious due to low probability of obtaining high-producing clones from a pool of transfected cells. Usually, many rounds of MTX selection to amplify the transgene and screening of over several hundred individual clones are required to obtain high-producing cells. Here, we present a novel concept to accelerate gene amplification through cell-cycle checkpoint engineering. In our knowledge, there is no previous report which focused on controlling cell cycle checkpoint to enhance the efficiency of DHFR gene amplification system.

EXPERIMENTAL APPROACH:
A small interfering RNA (siRNA) expression vector against Ataxia-Telangiectasia and Rad3-Related (ATR), a cell cycle checkpoint kinase, was transfected into Chinese hamster ovary (CHO) cells. The effects of ATR down-regulation on gene amplification and productivity in CHO cells producing green fluorescent protein (GFP) and monoclonal antibody (mAb) were investigated.

RESULTS AND DISCUSSION:
The ATR down-regulated cells showed up to 6- fold higher ratio of GFP-positive cells than that of the control cell pool, and had about 4- fold higher specific productivity and 3- fold higher volumetric productivity as compared to the control cell pool during the construction of mAb-producing cells. ATR down-regulated cells showed much faster increase of transgene copy number during gene amplification process via methotrexate (MTX) treatment in both GFP- and mAb- producing cells. Our results suggest that a pool of high producing cells can be more rapidly generated by ATR down-regulation as compared to the conventional gene amplification method by MTX treatment. This novel method is a promising approach to reduce the duration and labor in the process of cell line construction.

Tuesday 25th of June- 10h39
021

ENGINEERING A MAMMALIAN CELL LINE TOOLBOX THAT EXHIBITS MULTIPLE PRODUCTIVITY AND PRODUCT QUALITY PROFILES

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KEY WORDS:
HOST CELL ENGINEERING / PRODUCT QUALITY / PRODUCTIVITY

BACKGROUND AND NOVELTY:
Although the simplicity of having a single, well-characterized host upon which to initiate cell line development has many advantages, a one size fits all approach does have drawbacks. The range of product quality attributes achievable by a particular host will be limited by its intrinsic genotype and phenotype, which may not overlap with the optimum profile for a given therapeutic. The host proteome, particularly as it relates to folding and trafficking pathways, may differ in a manner that could influence the ability to effectively express some therapeutics. With this in mind, we have begun engineering a "toolbox" of different CHO and HEK293 cell lines to make them suitable hosts for the manufacturing of biologics.

EXPERIMENTAL APPROACH:
With the increased sophistication of engineering tools enabling precise genome editing now at our disposal, we endeavored to create and characterize modified mammalian hosts with tailored made phenotypes. This work describes the initial three step process in which new candidate hosts were i) adapted and evolved for enhanced performance in the platform process, ii) engineered for effective selection and amplification phases, and iii) subsequently auditioned with model proteins to assess the resulting diversity of the hosts. Particular interest was paid to individual engineered host growth rate, productivity and product quality attributes.

RESULTS AND DISCUSSION:
Across the different host cell lines generated and characterized, we saw variations in the ability to express the model proteins, frequency of potentially immunogenic glycans such as alpha-galactose and hydroxylated sialic acid, and different propensities for aggregation. This supports our original premise that each engineered host could contribute unique growth, productivity and product quality attributes resulting in a "cell line toolbox". We believe that moving forward this host cell toolbox affords us far greater flexibility and capabilities than a single host.

Tuesday 25th of June- 11h30
022

MULTI-GENE ENGINEERING OF MAMMALIAN CELL METABOLISM: WALKING THE STEPS TOWARDS HYPERPRODUCTIVITY

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KEY WORDS:
METABOLIC ENGINEERING / MULTI-GENE MANIPULATION / MAMMALIAN CELLS

BACKGROUND AND NOVELTY:
Mammalian cell metabolic engineering holds the potential to develop high-producing hosts for the manufacture of complex biopharmaceuticals. In 2007, G. Seth and colleagues conceptualized hyperproductivity as the orchestrated combination of superior attributes from different biochemical pathways [1]. But metabolic manipulation involves labor-intensive steps, from the introduction of the target gene to the isolation and characterization of the candidate clones, turning multiple manipulations extremely difficult. Multi-gene engineering has typically been restricted to 2-3 genes, with the most outstanding achievement reporting up to 3 siRNAs and 3 transgenes off a single genetic platform [2]. In this work, we experimentally challenge the theoretical concept of hyperproductivity to improve a mammalian cell host producing a recombinant enveloped virus, by the manipulation of more than 30 genes.

EXPERIMENTAL APPROACH:
A novel method was implemented for high-throughput screening of hundreds of clones in the early stages of cloning – single step cloning-titration method – allowing for fast isolation of the high-producing phenotypes in a metabolically engineered population. Assisted by a previous functional genomics study on the networks recruited when establishing a producer cell line, more than 30 candidates, including metabolic and regulatory genes, were chosen for manipulation. Targeted pathways included energy generation, oxidative stress, apoptosis, protein processing, lipid biosynthesis and nucleotide metabolism. Manipulated genes span across cytosol, mitochondria and endoplasmic reticulum.

RESULTS AND DISCUSSION:
When single genes were used, specific productivity raised up to 20-fold increase. Genes found to yield high-producing phenotypes are now being combined and the resulting clones characterized. The diversity of metabolic pathways targeted in this study is likely to be of relevance to several biopharmaceuticals produced in mammalian hosts, including complex proteins and other types of recombinant viruses.

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MONITORING INTRACELLULAR REDOX CHANGES IN BIOTECHNOLOGICALLY RELEVANT MAMMALIAN CELL LINES WITH GENETICALLY ENCODED FLUORESCENT BIOSENSORS

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KEY WORDS:
BIOSENSOR / REDOX / CHO-K1 / HEK-293 / HT-29

BACKGROUND AND NOVELTY:
Cellular functions such as DNA replication and protein translation are influenced by changes in intracellular redox homeostasis. Recently, redox-sensitive variants of the green fluorescent protein (roGFP2 and rxYFP) have been developed [1-3]. The biosensors were engineered to equilibrate with the intracellular pool of oxidized and reduced glutathione, the major redox buffer of most eukaryote, allowing the in situ and time-resolved analysis of the cellular redox state. The monitoring of intracellular redox changes can be useful to guide intervention strategies aimed at optimizing cell production processes. The goal of this work was to generate and characterize stable cell lines expressing a redox biosensor.

EXPERIMENTAL APPROACH:
CHO-K1-hGM-CSF (CHO-k1 expressing human granulocyte macrophage colony-stimulating factor), HEK293 and HT-29 cells were lipotransfected with pcDNA3.1-rxYFP plasmid. Cells were selected with zeocin or geneticin and cloned using a MoFlo cell sorter. The functional analysis of the biosensor was performed by flow cytometry. The growth, metabolism and productivity of the reporter CHO-K1-hGM-CSF-YFP and the parental cell line were compared in batch cultures.

RESULTS AND DISCUSSION:
All reporter cell lines displayed a sensitive and reversible response to different redox stimuli (H2O2, pro-oxidant and reducing agent). Cell density, glucose consumption, lactate and rhGM-CSF production were not affected by the introduction of the sensor in CHO-K1-hGM-CSF cells. Prior to entrance to the late log-phase (i.e. growth retardation) the biosensor revealed a significant and sustained shift to a more oxidative intracellular milieu. This suggests that the biosensor is capable to detect early metabolic deficiencies that alter the cellular redox balance in batch culture systems.
Ongoing work aims to test different additives and replenishment strategies to restore the cellular redox homeostasis to increase cell mass and productivity.

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The rxYFP-gene was a kind gift from Dr. G. Pani (Istituto di Fisica, Universitá Cattolica S. Cuore, Italy).

OTHER INFORMATION
K. Perelmuter and I. Tiscornia contributed equally to this work. This work was supported by Agencia Nacional de Investigación e Innovación, Uruguay (project FMV_2009_2617).

A COMPREHENSIVE VIEW ON AN OLD METABOLITE: LACTATE

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KEY WORDS:
LACTATE METABOLISM / MITOCHONDRIAL ACTIVITY / CELL CULTURE MEDIUM

BACKGROUND AND NOVELTY:
In animal cell culture processes, lactate belongs since years to the most intensively monitored metabolites. Lactate can strongly impact the productivity, mainly by influencing the culture pH. In bioreactors, the pH drop induced by excessive lactate production can be neutralized by base addition. However, in diverse non-regulated culture systems, the lactate accumulation can be fatal for the culture performances as soon as it causes the pH to drop below critical physiological values. Conversely, a moderate lactate accumulation at the beginning of the batch culture might be desirable, since rapid cell growth requires a highly active energy metabolism, which is characterized by aerobic glycolysis or "Warburg effect", first described as a criterion to distinguish fast growing cancer cells from normal tissue.

EXPERIMENTAL APPROACH:
In a comparative study involving several CHO clonal cell lines grown under various conditions we noticed different lactate profiles. In further tests under specific culture conditions, each clone generated its distinctive lactate profile. Additional data on a few model clones correlated the lactate profiles to the mitochondrial activity and the expression levels of certain genes such as aralar1 and timm8a. The lactate profiles of clones could be easily altered by feeding certain media components. Moreover we demonstrated that the culture medium can strongly affect the lactate profile as well as the expression of aralar1, which is known for its role in energy metabolism.

RESULTS AND DISCUSSION:
Our data clearly confirm that multiple strategies, including clone screening, medium composition change and cell line genetic engineering, can be employed to obtain a process in which cells switch their metabolism after a few days and start to consume lactate.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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Tuesday 25th of June- 12h22
025

COMPREHENSIVE UNDERSTANDING OF HEPARAN SULFATE PROTEOGLYCAN BIOSYNTHESIS IN CHO AND HEK293 CELLS

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KEY WORDS:
TRANSIENT GENE EXPRESSION / HEPARAN SULFATE PROTEOGLYCAN / ENDOCYTOSIS / CHO / HEK293

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK293) cells are the most popular host cells for transient production of therapeutic proteins. These host cells require high transfection efficiency in order to enhance productivity. While most mammalian cells express heparan sulfate proteoglycans (HSPGs) on the cell surface, recent studies have shown the close relationship between HSPGs and gene delivery via endocytosis. Albeit many researchers have revealed the direct involvement of HSPGs in transfection, metabolic process of HSPG biosynthesis in CHO and HEK293 cells is yet unknown.

EXPERIMENTAL APPROACH:
In this study, we performed immunofluorescence staining and detection of mRNA to demonstrate the biosynthesis, secretion, and degradation of HSPG in CHO DG44 and HEK293 EBNA cells.

RESULTS AND DISCUSSION:
The localization pattern of HSPG in both cells changed simultaneously with cell growth. The time course study and immunofluorescence showed that the synthesis of HSPG increased on day 2 after plating and the secretion and/or fading of HSPG became significant on day 4 after plating. Flow cytometry analyses showed fluctuated patterns possibly due to continuous biosynthesis-secretion-degradation circulation. The relative mRNA amount of 15 genes related with each step of HSPG biosynthesis showed parallel mode with immunofluorescence results. The expression level of enzymes related with glycosaminoglycan chain modification was especially high in both cells. In addition, overall mRNA expression level in HEK293E cells was higher than CHO DG44 cells. Furthermore, heparanase assay suggested that the gradual degradation of HSPG is related with the increased heparanase activity in the cells and media.
Taken together, our data show the relationship between morphological changes of HSPGs and mRNA expression level during the culture in CHO DG44 and HEK293E cells. This study provides clues to enhance the transfection efficiency in transient gene expression system by engineering cell surface HSPGs.

Wednesday 26th of June- 10h10
026

BEAT™ THE BISPECIFIC CHALLENGE: A NOVEL AND EFFICIENT PLATFORM FOR THE EXPRESSION OF BISPECIFIC IGGS

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KEY WORDS:
BISPECIFIC ANTIBODY / HETERODIMERIC IGG / BEAT / T CELL RECEPTOR / CHO

BACKGROUND AND NOVELTY:
The binding of two biological targets with a single IgG-based molecule is thought to be beneficial for clinical efficacy. However the technological challenges for the development of a bispecific platform are numerous. While correct pairing of heterologous heavy and light chains (HC and LC) can be achieved by engineering native IgG scaffolds, crucial properties such as thermostability, effector function or low immunogenicity should be maintained. The molecule has to be expressed at industrially relevant levels with a minimum fraction of contaminants and a scalable purification approach is needed to isolate the product from potentially complex mixtures. This presentation will introduce a novel bispecific platform based on the proprietary BEAT technology (Bispecific Engagement by Antibodies based on the T cell receptor) developed by Glenmark and will present the CLD strategy used for stable clone generation.

EXPERIMENTAL APPROACH:
The challenge of HC heterodimerization is solved by mimicking the natural association of the heterodimeric T-cell surface receptors and between two CH3 domains of IgG. LC mispairing is avoided by replacing one Fab arm of the bispecific IgG by a scFv. The protein A binding site of the HC is abrogated to facilitate the isolation of the BEAT-antibody by affinity chromatography.

RESULTS AND DISCUSSION:
The molecule shows good thermostability and low likelihood of immunogenicity. Stable cell lines are generated by co-transfection of proprietary expression vectors in CHO-S cells. The asymmetry of our bispecific format allows the characterization of the secretion profiles of generated clones using high throughput analytics based on the molecular weight. Using this approach, clones with volumetric productivity of several g/L and a high heterodimerization level (>90%) could be generated within 5 months. In summary, our platform combining the BEAT technology for heterodimerization and an efficient cell line selection strategy allows production of pure bispecific antibody at several g/L.

NEXT GENERATION BISPECIFIC ANTIBODY DESIGN AND THE INFLUENCE THEREOF ON PRODUCT YIELD AND STABILITY

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KEY WORDS:
BISPECIFIC ANTIBODY / MAMMALIAN EXPRESSION / CHO-S / STABILITY

BACKGROUND AND NOVELTY:
First-line cancer treatments such as surgical removal of tumours is highly invasive and if unbeknownst to patient or physician the cancer has spread to other organs, not an effective means of providing a therapeutic benefit; strengthening the need to develop targeted therapies capable of overcoming drug resistance and limiting the immunogenic effects associated with chemotherapeutics. One novel approach to circumvent this phenomenon includes the engineering of new antibodies capable of targeting cell surface antigens for which natural antibodies don't exist. Monoclonal antibodies (mAbs) provide a means to circumvent systemic drug administration when conjugated to drugs or radio-labels. Bispecific antibodies (BsAbs) can be engineered to target multiple antigens; whether these are cancer cell surface antigens, specific drugs or targets on a drug delivery nanoparticle; explaining why these next generation biomolecules have gained popularity in recent years.

EXPERIMENTAL APPROACH:
Development of processes to produce high levels of BsAbs has proved to be more complicated than standard production techniques required for mAb development owing to the lower expression levels of BsAbs and their inherent downstream instability. We have engineered a number of standard BsAb formats for production in a CHO-S expression system and will relate changes in BsAb structure to the total yield following chromatographic purification.

RESULTS AND DISCUSSION:
BsAbs are expressed at a significantly lower level than standard mAbs. Slight modifications at the BsAbs DNA level can results in two similar constructs having 6-fold differences in product yield. However, the same changes in the BsAb DNA sequence results in improved downstream and long-term stability of the product. Developing the mammalian expression and chromatographic techniques to enhance BsAb production is therefore a pivotal component in improving product yield and stability.

INVESTIGATING THE DETERMINANTS OF NOVEL-FORMAT ANTIBODY EXPRESSION IN CHO CELLS

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KEY WORDS:
EXPRESSION / AMINO ACID SEQUENCE / PHAGE DISPLAY / TRANSIENT HEK / WESTERN BLOTTING

BACKGROUND AND NOVELTY:
Recent innovations in antibody engineering have resulted in a new generation of novel-format antibody-based products, designed to improve natural antibody properties.[1] However these non-natural antibodies can be challenging for host cell production [2,3] and expressional bottlenecks may occur at any stage of expression, [2,3] which has an impact on the production and entry of novel-format antibodies onto the clinical market. There is growing evidence that amino acid sequence can impact on protein expression [2] but the molecular mechanisms that govern this observation are still poorly understood.

EXPERIMENTAL APPROACH:
In this study we generated a panel of novel-format antibodies based around a common monoclonal antibody to which we have attached a range of binding proteins generated through phage display technology against a common antigen and with limited variation in amino acid sequence at discrete areas of the binding protein. These novel format antibodies have been screened for expression in transient HEK cells and 20 sequences were progressed into stable CHO cells to perform in-depth molecular analyses of determinants of expression of this novel antibody format.

RESULTS AND DISCUSSION:
We have observed that limited amino acid variations (between 10-16% of the binding protein) can result in up to a 10-fold difference in expression in both HEK and CHO cells. Expression in HEK cells was generally, but not always, a good predictor of expression in CHO cells. Host cell growth and viability characteristics were not adversely affected by transfection with poor expression constructs and there was no relation between poor expression and functionality of the novel-format antibody. Intracellular and extracellular western blotting has enabled categorisation of profiles that correlate with expression. These results will give insight into the relation between amino acid sequence and expression in mammalian cells and have an impact on the development of engineered antibodies for the clinical market.

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Wednesday 26th of June- 10h54
029

A QUANTITATIVE AND MECHANISTIC MODEL FOR MONOCLONAL ANTIBODY GLYCOSYLATION AS A FUNCTION OF NUTRIENT AVAILABILITY DURING CELL CULTURE

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KEY WORDS:
MONOCLONAL ANTIBODY GLYCOSYLATION / QUALITY BY DESIGN / MATHEMATICAL MODELING / BIOPROCESSING

BACKGROUND AND NOVELTY:
Monoclonal antibodies (mAbs) are one of the leading products of the pharmaceutical industry. All approved mAbs contain a consensus N-linked glycosylation site on their constant fragments, and it has been widely reported that the complex carbohydrates (glycans) bound to these sites have great influence on the safety and efficacy of these molecules. It has also been reported that numerous bioprocess conditions directly impact the composition and distribution of glycans bound to mAbs. To address these issues, we have defined a mathematical model that mechanistically and quantitatively describes the glycosylation profiles of mAbs as a function of nutrient availability during cell culture. Such a model can be used for bioprocess design, control and optimization, thus facilitating the manufacture of mAbs with built-in glycosylation-associated quality under QbD guidelines. To our knowledge, this is the first mathematical model that quantitatively relates bioprocess conditions with mAb glycosylation.

EXPERIMENTAL APPROACH:
The mathematical model links extracellular nutrient availability with nucleotide sugar (NSD) metabolism which, in turn, feeds into a previously published model for Golgi N-linked glycosylation [1]. Experimentally, murine hybridoma cells (CRL-1606, ATCC) were cultured and typical data was collected. The intracellular NSD pools were extracted using perchloric acid (PCA) and quantified using a chromatographic method (HPAEC) that allows for quantification of 8 NSDs and 4 nucleotides in under 30 minutes. Finally, the mAb glycan profiles were obtained using MALDI TOF mass spectrometry.

RESULTS AND DISCUSSION:
Time-courses for all data were produced, including the profiles for all eight NSDs and four nucleotides. The mathematical model reproduces all the experimental data accurately. For validation, simulations were performed under different conditions of nutrient availability. These simulations show that the model has the ability of reproducing previously reported phenomena, such as production of high-mannose glycans under glutamine starvation [2].

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Wednesday 26th of June- 12h05
030

ELECTRICALLY MODULATED ATTACHMENT AND DETACHMENT OF ANIMAL CELLS CULTURED ON AN ITO ELECTRODE

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KEY WORDS:
ELECTRICAL MODULATION / CELL ATTACHMENT / CELL DETACHMENT / HELA CELL / DEEP-SEA FISH CELL

BACKGROUND AND NOVELTY:
Micropatterning techniques of adhesive animal cells have been reported by numerous groups and fall into 6 major classifications. There are 1) photolithography, 2) soft lithography, 3) ink jet printing, 4) electron beam writing, 5) electrochemical desorption of self-assembled monolayers, and 6) dielectrophoresis. However, these six cell micropatterning techniques cannot modulate both the attachment and detachment of animal cells iteratively at the same positions. The purpose of this study was to develop modulation methods for the attachment and detachment of specifically positioned adhesive animal cells cultured on an electrode surface with the application of a weak electrical potential.

EXPERIMENTAL APPROACH:
A patterned indium tin oxide (ITO) optically transparent working electrode was placed on the bottom of a chamber slide with a counter- (Pt) and reference (Ag/AgCl) electrode. The ITO patterning was formed by a reticulate ITO region and arrayed square glass regions of varying size. Using the 3-electrode culture system, the author succeeded in modulation of the attachment and detachment of animal cells on the working electrode surface.

RESULTS AND DISCUSSION:
Animal cells suspended in serum or sera containing medium were drawn to and attached on a reticulate ITO electrode region to which a +0.4-V vs. Ag/AgCl-positive potential was applied. Meanwhile, the cells were successfully placed on the square glass regions by –0.3-V vs. Ag/AgCl-negative potential application. Animal cells detached not only from the ITO electrode but also from the square glass regions after the application of a ±1.0-V vs. Ag/AgCl, 9-MHz triangular wave potential in PBS(–) for 30–60 min. Triangular wave potential-induced cell detachment is almost completely noncytotoxic, and no statistical differences between trypsinization and the high frequency wave potential application was observed in HeLa cell growth.

REF-A001

PRODUCTION OF MONOCLONAL ANTIBODY, ANTI-CD3 BY HYBRIDOMA CELLS CULTIVATED IN BASKET SPINNER UNDER FREE AND IMMOBILIZED CONDITIONS

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KEY WORDS:
PRODUCTION / MONOCLONAL / BASKET SPINNER / IMMOBILIZATION / HYBRIDOMA

BACKGROUND AND NOVELTY:
Recently, packed-bed bioreactors have been used for the cultivation of a wide range of cell lines and for the production of a large variety of pharmaceuticals including MAbs. Packed-bed bioreactor depends on the immobilization of cells within a suitable stationary matrix (the bed). Packed-bed bioreactors also have the advantage of being capable of generating high cell densities having a low free-cell concentration in suspension; hence, simplifying downstream processing.

EXPERIMENTAL APPROACH:
Hybridoma cells (OKT3), producing IgG2a monoclonal antibodies against CD3 antigen of human T lymphocytes were adapted to serum free medium. The specificity of the produced MAbs was performed by indirect immunofluorescence staining of T lymphocytes from peripheral blood followed by flowcytometeric analysis. Continuous production of MAb was performed in Basket Basket through cell immobilization on Fibra-Cel disks.

RESULTS AND DISCUSSION:
The results obtained showed that upon using flow cytometry and the fluorochrome-conjugated secondary antibody attached specifically to the supernatant MAb from the cells adapted to serum free medium succeeded in sorting 76.8% of the gated cells (lymphocytes), confirming the binding of MAb of the adapted cells to CD3 positive lymphocytes. This means that stable hybridoma cells were successfully adapted to grow under serum free conditions. Upon cultivating the cells in backed spinner basket, the MAb titer increased in each successive batch to reach to 298.5 mg.L-1 after 216 h. This might be due to the protection of the cells against shear stress and air/O2 sparging through their immobilization on the micro-carriers, promoting the use of serum- or protein-free medium. Moreover, the micro-carrier is designed to ensure sufficient nutrient supply and also to remove toxic metabolites. On the other hand, the rate of glucose consumption and lactate production increased for each repeated batch, which explains the decrease in batch time and reflects the better physiological state of the cells.

REF-A002

EVALUATION OF A NEW 2D ROCKING-TYPE SINGLE-USE BIOREACTOR FOR STREAMLINED CELL EXPANSION

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KEY WORDS:
CELL EXPANSION / SINGLE-USE BIOREACTOR / SINGLE-USE SENSOR

BACKGROUND AND NOVELTY:
Multi-stage seed trains are typically used to expand a cell population to seed large-scale bioreactors. Co-development of a novel 2-D rocking single-use bioreactor that has a wide range of working volumes will be presented. The rocking mechanism enables high oxygen transfer rates at low shear stress.

Conventional seed trains consist of multiple containers of different types such as spinner flasks, shake flasks, and single-use bioreactors of different sizes. Each device requires its own equipment, incubators and/or platforms contributing to the highly manual and time consuming nature of these operations. Repeated manipulations, also increase the risk of contamination.

EXPERIMENTAL APPROACH:
Test expansions were conducted using the novel bioreactor equipped with a disposable bioreactor bag designed to accommodate a range of culture volumes from 160 mL to 25 L as the cells grow. The new bag was also fitted with integrated single-use sensors to allow better control of cell culture conditions and a new concept for perfusion with an integrated membrane to achieve higher cell concentrations.

RESULTS AND DISCUSSION:
Cell growth and sensor data from the test expansions will be presented and compared with results from a more conventional multi-container seed train. The potential for using the sensor data to control cell culture conditions and to automate the expansion steps will be discussed.

CHARACTERIZATION OF SOY AND WHEAT GLUTEN HYDROLYSATES PROMOTING VERO CELLS GROWTH UNDER ANIMAL COMPONENT FREE CONDITIONS

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KEY WORDS:
SOY AND WHEAT HYDROLYSATES / VERO

BACKGROUND AND NOVELTY:
We developed an animal component free medium named IPT-AFM that sustains Vero cells growth and rabies virus production in stirred bioreactor. This medium contains plant hydrolysates, namely soy (Hypep 1510) and wheat gluten hydrolysates (Hypeps 4601 and 4605) (Rourou et al., 2009). These peptones were shown to promote cell attachment and growth. In a previous work, we fractionated these hydrolysates in order to identify and isolate the peptides showing a positive effect on Vero cells adhesion, attachment and growth (Rourou et al., 2011). After Hypep 4605 removal, we demonstrated that that the performances of IPT-AFM were preserved.

Recently, there has been a tremendous research interest in the production, characterization, and evaluation of bioactive ingredients present in plant-derived products. Their positive impact on body functions or conditions is highly sought.

EXPERIMENTAL APPROACH:
The aim of this work is to assess the global chemical composition such as size distribution, amino acids and carbohydrates composition; and the biological activities of the fractions previously isolated and that had shown a positive effect on Vero cell growth.

RESULTS AND DISCUSSION:
Peptides have certain bio-functionalities and may therefore fulfil therapeutic roles in body systems. For this reason, to assess the biological activities of the selected fractions of soy hydrolysates, we will particularly focus on the ACE inhibitory activity (antihypertensive), antioxidant and anticancer activities. In addition, we will apply the Tricine-SDS-PAGE to analyse the different fractions issues from both hydrolysates.

Then fractions that exhibit a biological activity will be further fractionnated to isolate the peptides. The biological activities of these peptides will be also tested.

RAPID MHC CLASS II PROTEIN PRODUCTION BY TRANSIENT GENE EXPRESSION OF INSECT CELLS

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KEY WORDS:
INSECT CELLS / TRANSIENT GENE EXPRESSION / MHC II MOLECULES / HIGH EFFICIENT

BACKGROUND AND NOVELTY:
Recombinant major histocompatibility complex (MHC) class II proteins have important uses in basic and clinical immunology. They are commonly expressed from stable Drosophila S2-derived cell lines with volumetric productivities of 10-15 mg/L.

EXPERIMENTAL APPROACH:
Here we describe a more rapid approach to high-level expression of MHC II molecules by large-scale transient gene expression (TGE) of suspension-adapted insect cells. The MHC II and chain genes were cloned into separate expression vectors under the control of either an inducible or a constitutive promoter, and the plasmids were co-transfected into suspension-adapted High Five™, Sf9, or Schneider S2 cells using a chemical reagent for DNA delivery.

RESULTS AND DISCUSSION:
Volumetric yields of over 200 mg/L were obtained within 4 days for some MHC class II proteins. Our results demonstrate a simple, fast and low-cost approach to express recombinant MHC class II molecules.

REF-A005

CRYOPRESERVATIVE SOLUTION USING RAKKYO FRUCTAN AS CRYOPROTECTANT

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KEY WORDS:
FRUCTAN / RAKKYO / CRYOPRESERVATION / DMSO / FBS

BACKGROUND AND NOVELTY:
Cryopreservation of the cells allows great flexible application for cell therapy, as well as industrial production of biologics such as antibody therapeutics. Conventionally, cryopreservative solution contains both of fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) as cryoprotectants. However, both of them have problems.

FBS frequently induces differentiation of stem cells and so it should not be used for cell therapy. Additionally, FBS has serious concern about zoonotic infections such as abnormal prions, pathogen of bovine spongiform encephalopathy (BSE), indicating necessity of FBS-free cryopreservative solution. DMSO has cytotoxicity and often induces stem cells to differentiate. Therefore, it is necessary to reduce the concentration of DMSO in cryoprotectant solution.

In this study, we report that rakkyo fructan, plant-derived polysaccharide, significantly improved the viability of the cells frozen in DMSO-free solution.

EXPERIMENTAL APPROACH:
Cells were collected by centrifugation, removed the culture supernatant and then suspended in PBS containing rakkyo fructan or in FBS containing DMSO as positive control. They were transferred to freezing tubes, placed in a BIOCELL container, frozen and stored at -80 °C for several days. Stored cells were defrosted at 37 °C rapidly and removed the freezing media. They were cultured in each culture media again. The defrosted cells were stained with trypan blue exclusion method and counted with hemocytometer.

RESULTS AND DISCUSSION:
The cells stored in rakkyo fructan solution successfully survived after deep freezing-store and thawing process. After frozen and thawed in rakkyo fructan solution, CHO-DP12 cells and HepG2 cells maintained the growth rate and kept high protein productivity.

We successfully developed serum-free freezing media using rakkyo fructan. The freezing media using rakkyo fructan will be extensively used to protect various animal cells against freezing stress.

REF-A006

DEVELOPMENT AND PERFORMANCE QUALIFICATION OF A NEW SINGLE USE BIOREACTOR SYSTEM

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KEY WORDS:
CELL EXPANSION / SINGLE-USE BIOREACTOR / SINGLE-USE SENSOR

BACKGROUND AND NOVELTY:
A newly developed single use bioreactor system, the Pall XRS 20 Bioreactor system, consists of a rocking platform which holds a pre-sterilised single use biocontainer. Fluid motion essential for gas transfer and liquid-liquid mixing within the biocontainer is achieved using two motors mounted on opposite axes. This enables motion on both the x and y axis (bi-axial agitation). Each bioreactor system includes a control tower to allow the operator to select the agitation rate and rock angles, as well as control the temperature, pH and dissolved oxygen content of the biocontainers liquid content.

EXPERIMENTAL APPROACH:
Our approach included design of experiment to refine agitation parameters for optimized mixing and mass transfer properties, followed by multiple cell culture batch and fed-batch experiments to compare cell culture performance in the new design versus performance in conventional rocker-style systems.

RESULTS AND DISCUSSION:
We present how the introduction of bi-axial agitation in to bench scale single-use bioreactors can significantly improve CHO cell culture performance over conventional rocker platforms. We will present examples of how the bi-axial agitation during high cell density cell culture maintained all the benefits of a low shear environment yet significantly reduced mixing time and increased O2 transfer rate with no accumulation of CO2. For fed-batch process this resulted in consistently reproducible increases in cell titre, IVCC and monoclonal antibody titre; whilst maintaining an identical product quality profile compared to results from conventional rocker systems.

EFFECT OF REDOX POTENTIAL ON ANTIBODY GLYCOSYLATION IN MAMMALIAN CELL CULTURES

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KEY WORDS:
CELL CULTURE / MONOCLONAL / CAMELID / REDOX / GLYCOSYLATION

BACKGROUND AND NOVELTY:
Consistent and proper glycosylation is very important in ensuring Mabs (monoclonal antibodies) efficacy and effectiveness. The consensus glycan in an immunoglobulin has often been related to its position in the interstitial space between the disulfide bonded CH2 domains of two heavy chains. This can reduce the accessibility of glycosyl-transferase enzymes particularly the galactosyltranfersase that adds the terminal galactose to form the G1 or G2 structures. Lowering culture redox potentials (CRP) may disrupt Mab interchain disulfide bonds and lead to different glycan profiles. Using CHO and NS0 cell cultures, several Mabs were analysed for their glycan profiles; including 2 humanized IgG1s and a humanized camelid Ig in the presence of a reducing agent.

EXPERIMENTAL APPROACH:
Cell cultures of CHO and NS0 were subjected to various concentrations of reducing agent (dithiothreitol;DTT) and monitored daily for growth parameters and redox potential. Using HILIC-HPLC methods, shifts in the GI (Galactosylation Index) were monitored in the presence of DTT. In addition to glycan profiling, IgG1 from NS0 cultures were radiolabelled to determine the assembly pathway and changes in pathway intermediates in the presence of the reducing agent.

RESULTS AND DISCUSSION:
The GI was decreased in NS0-IgG1 cultures by as much as 35% in those cultures with lower CRP. In contrast, CHO-IgG1 cultures had no change in GI and the camelid Ig exhibited a 16% increase in GI. The autoradiographs of the protein A purified intracellular NS0-IgG1 verified an assembly pathway of HC HC2 LCHC2 LC2HC2. Densitometry analysis of assembly intermediates showed that the ratio of heavy chain dimer to heavy chain monomer increased over time within the reducing agent cultures. A correlation between redox potential, GI shifts and assembly intermediates is suggested by the data for this particular IgG1. This could have wide ranging implications for process development activities and lead to control mechanisms that influence glycan profiles of Mabs.

VIRAL VECTOR PRODUCTION IN THE INTEGRITY™ ICELLIS® DISPOSABLE FIXED-BED BIOREACTOR FROM BENCH-SCALE TO INDUSTRIAL SCALE

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KEY WORDS:
VIRAL VECTORS / SINGLE-USE BIOREACTOR / TRANSIENT TRANSFECTION / RECOMBINANT VIRUSES / SCALE-UP

BACKGROUND AND NOVELTY:
Recombinant viruses (e.g. lentivirus and adeno-associated-virus) can be used as human gene therapy vectors. They are mainly produced in adherent cell cultures (e.g. HEK293T, A549, VERO) in Roller Bottles (RB) or multiple-tray-stacks using either transient transfection (e.g PEI, PO4 precipitation) or infection (e.g. recombinant viruses) strategies. Therefore, iCELLis® bioreactors offer a new production alternative with stronger process controls and ease of scale-up.

The iCELLis bioreactor from ATMI LifeSciences is designed for adherent cell culture applications. Cells grow on microfibers carriers packed in a fixed-bed providing up to 500m^2 of growth surface area in a small reactor volume. Environmental conditions, combined with the large growth surface area in the iCELLis yields high cell productivity.

EXPERIMENTAL APPROACH:
First, a mirrored approach of the previous production in RB or CF was set-up in the small scale iCELLis bioreactor. Transfection/infection efficiency through the fixed-bed of the iCELLis system is evaluated by Flow Cytometry (measuring the expression of protein marker). At harvest, extracellular viral vectors are cultivated in the cell culture medium. In the case of intracellular vectors, cell disruption is carried out directly in the bioreactor by physico-chemical methods.

RESULTS AND DISCUSSION:
Here we present results of AAV and paramyxovirus production in the iCELLis nano versus classical culture recipients. These results indicated higher titer for AAV (5.108 vg/cm2) in small scale iCELLis versus 3.108 in Cells Stack-5 plates (CS, Corning) and 0.5-1 log higher titers than control (tissue culture flask) for paramyxovirus. Results of transient transfection processes (by PEI or PO4 precipitation) showed similar transfection levels in iCELLis than in CS. Results in iCELLis demonstrated that the system allows high biomass growth, regulation, and virus productivity with a minimum space requirement. The technology can be considered an efficient tool for the production of viral vectors.

REF-A009

CRYOPRESERVATIVE SOLUTION USING RAKKYO FRUCTAN AS CRYOPROTECTANT

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KEY WORDS:
VIRUS / SINGLE-USE BIOREACTOR / SCALE-UP

BACKGROUND AND NOVELTY:
Viral vaccines are usually produced by anchorage-dependent cells in static multitray systems, roller bottles or bioreactors with microbeads. However, these technologies do not enable process intensification as they involve many manual operations. In addition, microbeads-based processes require extensive development and expertise.
To enable process intensification, ATMI developed iCELLis®, a scalable range of disposable fixed-bed bioreactors that operate in perfusion mode. The fixed-bed accommodates up to 500m^2 of growth surface area in only 25 liters reactor volume. The fixed-bed is pre-packed with microfiber carriers to avoid extensive process development related to microbeads. It can be inoculated at a very low cell density which simplifies seed train.

EXPERIMENTAL APPROACH:
Here we present the scale-up of MDBK, Vero and HEK293 processes from iCELLis benchtop bioreactor to production units. Scaling-up with iCELLis systems is quick and similar to that of chromatography columns. As the bioreactor scale increases, the fixed-bed height remains constant while the diameter increases. The cell culture data presented here illustrates the success of this scale-up approach.

RESULTS AND DISCUSSION:
Existing multitray system processes were transferred to small scale iCELLis by keeping same culture parameters (pH, DO & t°) and identical ratios for cells/surface and media/cells. Next came evaluation and optimization of cell culture conditions: compaction of carriers, the linear speed through the fixed-bed and the perfusion rate. When these conditions guaranteed homogeneous cell distribution and good viral productivity, the process was directly scaled to an industrial level. At such scale, we obtained a cell biomass of 1.75x1012 Vero cells (500m^2), 2.2x1011 MDBK cells (133m^2) and 4.5x1011 HEK293cells (133m^2).
The study demonstrated that iCELLis bioreactors simplify viral production processes and that linear scale-up in iCELLis is easily developed from 0.53m2 to 500m2 in less than one year.

REF-A010

A COMPARATIVE STUDY OF SINGLE-USE WAVE-MIXED AND STIRRED BIOREACTORS IN INSECT CELL/BEVS-BASED PROTEIN EXPRESSION AT BENCHTOP SCALE

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KEY WORDS:
INSECT CELLS / BEVS / PROTEIN EXPRESSION / SINGLE-USE BIOREACTORS

BACKGROUND AND NOVELTY:
The cultivation of insect cells in conjunction with the Baculovirus Expression Vector System (BEVS) is considered a promising source for new vaccine candidates as well as protein complexes. Wave-mixed and stirred bioreactors are currently employed for the cultivation of various insect cells and expression of their protein products. However, wave-mixed bioreactors have been reported several times as recommended cultivation systems for insect cell-based protein production processes, because of their low shear mixing principle which results in minimized foam formation. Still, no comparative study describing protein expression in wave-mixed and stirred single-use bioreactors has been published so far. This study investigates and compares the expansion of Spodoptera frugiperda-9 (Sf-9) suspension cells as well as the expression of a model protein, the secreted alkaline phosphatase (SeAP).

EXPERIMENTAL APPROACH:
Optimum infection and production conditions were determined in high-throughput screening systems such as the orbitally-shaken BioLector (m2p-labs) or TubeSpin® Bioreactor 50 (TPP). Growth and production studies were carried out in the wave-mixed BIOSTAT CultiBag RM (27 °C, 19 - 32 rpm, 0.1 vvm, pO2 50 %, pH 6.2) and stirred UniVessel SU (27 °C, 180 rpm, 0.1 vvm, pO2 50 %, pH 6.2) single-use bioreactor from Sartorius Stedim.

RESULTS AND DISCUSSION:
Best protein expression in screening experiments was obtained when using a cell count of infection (CCI) of 2 x 10E6 cells/mL, a multiplicity of infection (MOI) of 0.01 and a time of harvest (TOH) of 144 h. Whereas growth experiments in the two benchtop bioreactors resulted in high cell densities exceeding 10 x 10E6 viable cells/mL, strong foaming in the UniVessel SU required the addition of an antifoam agent, which was not required with the BIOSTAT CultiBag RM. Production experiments in the two bioreactors revealed comparable protein expression with respect to protein quality and activity. Maximum SeAP activities of up to 64 U/mL were achieved.

REF-A011

BIOPROCESS DEVELOPMENT OF DROSOPHILA S2 CELL CULTURE FOR THE EXPRESSION OF RABIES VIRUS GLYCOPROTEIN

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KEY WORDS:
DROSOPHILAS2CELLS/RABIESVIRUS/BIOPROCESS/RECOMBINANT PROTEIN

BACKGROUND AND NOVELTY:
Here we report a bioprocess development using Drosophila melanogaster Schneider 2 (S2) cells to produce the rabies virus glycoprotein (RVGP). The S2 cell system offers suitable bioprocess conditions for generating high-level expression of functional membrane proteins. It is relatively easy and safe to handle and are scalable. Acquired knowledge on biology and engineering of S2 cells opens well based conditions for production of recombinant proteins.

EXPERIMENTAL APPROACH:
We have constructed gene vectors with the hygromycin selection gene (H) in which the RVGP gene was inserted under the control of the metalothionein (Mt) promoter. After transfection, cell populations with (S2MtRVGP-H-his) and without (S2MtRVGP-H) His-tag amino acid motif were selected. The expression of RVGP was evaluated by qRT-PCR, flow cytometry, ELISA and western-blotting. Protocols for cell cultures in scalable bioreactors were developed and batches of RVGP were produced and purified. The ability of S2 cells derived RVGP to induce immune response and protect mice against an experimental rabies virus challenge were investigated.

RESULTS AND DISCUSSION:
High RVGP expression level could be detected in both S2 cell populations (~ 52 % of RVGP positive cells with ~ 4 micrograms of RVGP per 1E7 cells). RVGP mRNA kinetic analysis by qRT-PCR enlightened the relationship between S2 cell growth and specific productivity, showing a peak of RVGP mRNA and RVGP synthesis at the transition to the stationary cell growth phase. Parameters for storage and lysis of cells bearing the RVGP were studied and a protocol of RVGP purification was developed based on His-tag affinity chromatography (HisTrap FF-FPLC) after membrane preparations by ultracentrifugation and solubilizing with OG. High levels of antibodies against RVGP (~ 4 EU/mL after 21 days of immunization) were found in immunized mice (3 weekly doses of 3 micrograms of RVGP each). Preliminary data show that RVGP immunization was capable of inducing protection against rabies experimental challenge (~ 90 % of mice survived a rabies virus challenge). Our data describe bioprocess optimization steps for high-level and biological active RVGP expression in stably transfected S2 cells.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-A012

EFFECT OF AMINO ACIDS ADDITION IN S2 DROSOPHILA MELANOGASTER CELLS CONTINUOUS CULTURE

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KEY WORDS:
INSECT CELLS / GLUTAMINE / CYSTEINE / CELL CONCENTRATION / BIOREACTOR

BACKGROUND AND NOVELTY:
S2 cells from Drosophila melanogaster have been used as expression systems for recombinant proteins. A major goal in these process is increase cell density in bioreactor, to obtain more product. In this study we added amino acids to a commercial medium in order to increase cell concentration in continuous cultures of S2 cells.

EXPERIMENTAL APPROACH:
A S2 cell population was used in this work, which expresses recombinant rabies virus glycoprotein (RVGP) gene under the control of constitutive actin promoter, obtained by co-transfection procedure (Yokomizo et al., 2007). The cells were grown in a Biostat B bioreactor, at 28 degrees Celsius, 90 rpm agitation frequency, and dissolved oxygen controlled at 30 % of air saturation. pH was only monitored. Medium used was SF900 II (Invitrogen) pure or supplemented with glutamine, a pool of (asparagine, proline, serine and cysteine), or cysteine only. Three different dilution rates (0.8, 0.5 and 0.2/day) were visited. In parallel, Schott shake flasks runs were performed, to test conditions.

RESULTS AND DISCUSSION:
With pure SF900 II medium at different dilution rates, cell concentration reached in steady states 26.4 E6 cell/mL, with residual concentration of glucose of circa 5.3 g/L and glutamine of 0.45 g/L. The addition of glutamine (1.7 g/L) or a pool of amino acids in the feed medium did not contribute to increase significantly cell concentration. However, addition of only cysteine (0.3 g/L) in the feed medium resulted in a 12% increase in cell concentration as compared to pure SF900 II medium. Also, specific glucose and glutamine consumption rates were reduced in this case. Thus, it can be concluded that in SF900 II the cysteine limited cell growth of S2 cells and the addition of asparagine, proline and serine have a negative effect in cell concentration.

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Acknowledgments:CNPq

REF-A013

DROSOPHILA MELANOGASTER S2 CELL CULTURES IN CLASSICAL AND DISPOSABLE FIXED BED ICELLIS BIOREACTORS

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KEY WORDS:
DROSOPHILA S2 CELLS / FIXED BED ICELLIS BIOREACTOR

BACKGROUND AND NOVELTY:
New generations of disposable bioreactors have a central place in recent developments in animal cell biotechnology. ATMI LifeSciences developed Integrity® iCELLis®, a scalable line of single-use high-cell-density bioreactors operating in perfusion mode. By combining the advantages of single-use technologies with the benefits of a fixed-bed system, iCELLis systems have been shown to be an important advance in terms of high productivity combined with ready to use bioreactor for academic and industrial use. Our aim in the present work has been to evaluate the growth of Drosophila melanogaster S2 cells in classical and disposable iCELLis bioreactors.

EXPERIMENTAL APPROACH:
An S2 cell population was selected after transfection of gene expression vectors carrying the cDNA encoding the rabies virus glycoprotein (RVGP) gene (Moraes et al., Biotechnology Advances 2012, 30:613-628). Cells were initially cultivated in 50 mL shake flasks in SF900II medium and inoculated into the bioreactors at 5x1E5 cells/mL. Cell cultures were performed in a 1L suspension bioreactor (BioFlo - New Brunswick) at 90 rpm and in a iCELLis Nano bioreactor (with fixed bed of 0.53 m^2 total available surface) (ATMI) at 400-700 rpm and with a 800 ml medium working volume. Medium exchanges were performed and comparable temperature (28ºC) and dissolved oxygen (50 %) parameters were used.

RESULTS AND DISCUSSION:
The data show the good productivity in terms of cells in both systems with a potential for further developments. S2 cell cultures attained concentrations higher than 1E7 cells/mL after 5 days, showing maximal specific cell growth of 1.056 d-1. Glucose and glutamine were regularly consumed indicating suitable metabolic state of the cells. Lactate was produced in low concentrations. The iCELLis system provides a ready to use and flexible system for cell cultivation. Beside its contribution to optimization approaches of productivity, the present study provides bioprocess conditions for further studies of recombinant proteins expressed in S2 cells.

REF-A014

SINGLE-USE TECHNOLOGIES SUPPORT CONTINUOUS PROCESSING IN BIOPRODUCTION

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KEY WORDS:
CONTINUOUS / PERFUSION / STEADY-STATE / BIOPRODUCTION

BACKGROUND AND NOVELTY:
Continuous processing (CP) in pharmaceutical production has been strongly encouraged of late by regulatory and engineering stakeholders alike (1). CP offers many advantages over such currently popular modes of animal cell culture as batch and fed-batch. It provides heightened processing consistency resulting in improved product uniformity while reducing intervention in process intermediates. Process capability can be heightened and activities or chemistries unavailable in batch presented. CP also provides advantages in facility design and build through reduced footprint and increased facility utilization. Finally it's on-line monitoring and real-time quality assurance supported makes it amenable to CQV and even parametric or real-time release initiatives (2).
(1) http://www.in-pharmatechnologist.com/Processing/Continuous-manufacturing-will-make-current-methods-obsolete-FDA-says
(2) http://www.bioresearchonline.com/doc.mvc/Continuous-Processing-In-Bioproduction-0001

EXPERIMENTAL APPROACH:
Centocor (now Janssen Biotech) has long been employing semi-continuous operations in upstream processes of approved biopharmaceutical manufacturing. Genzyme manufactures such products as Lumizyme in CHO-based perfusion culture. Their continued commitment to perfusion-based production is demonstrated by its recent expansion of such capacity at their Geel, Belgium plant. Practical implementation of the perfusion mode of culture has been facilitated of late by the increased process understanding in general, the ability to take many more real-time process measurements, as well as by appearance of improved process control technologies. A new contributor to the growing field of continuous processing is the rapid uptake of single-use technologies (SUT). Reviewed here are those specific features afforded by SUT that relate to continuous processing-specific PD and manufacturing.

RESULTS AND DISCUSSION:
Continuous processing operations are being implemented throughout the process train and these examples in pharmaceutical production can be thought of as 'building blocks' toward an untimely fully continuous manufacturing line. Equipment and systems supporting continuous processing in operations from seed-stock expansion to fill and finish are appearing. As perfusion-modified reactors are contiguously combined with such other enabling technologies as single-use mixers and storage systems, the design of closed, disposable and continuous manufacturing systems for biopharma is finally being accomplished.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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PRODUCTION AND PURIFICATION OF DIFFERENT SEROTYPES OF RECOMBINANT ADENO-ASSOCIATED VIRUS AS A VECTORED VACCINE AGAINST HEPATITIS E VIRUS

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KEY WORDS:
HEPATITIS E VIRUS / INSECT CELLS / BACULOVIRUS / ADENO-ASSOCIATED VIRUS

BACKGROUND AND NOVELTY:

Hepatitis E virus (HEV) infection is the major cause of acute hepatitis in Southeast and Central Asia and the second most important cause in the Middle East and North Africa. Currently, no commercial HEV vaccine is available. In the absence of an appropriate cell culture system for HEV propagation, HEV pseudocapsids (ORF2 protein) have been produced either in Escherichia coli or in insect cells and they have been shown to protect monkeys against virus challenge and to be effective in the prevention of natural HEV infection of humans. In this work, we investigated the development of a novel candidate vaccine against hepatitis E infection using adeno-associated virus (AAV) as a vector expressing the gene of the truncated capsid protein of HEV (aa 112-aa 660). rAAV will be produced in Sf9 cells using the baculovirus expression vector system.

EXPERIMENTAL APPROACH:

For this purpose, construction of recombinant baculoviruses (BacRep, BacCap for serotypes 2, 5 & 6 and BacITRHEVORF2) were performed and viral stocks were amplified in Sf9 cells. To improve rAAV 2, 5 and 6 production in Sf9 insect cells, viral production was optimized in Erlenmeyer flask using the experimental design approach. We analyzed the effects of the following factors: initial cell density, time of infection, temperature and individual Multiplicity of infection (MOI) of the three or dual (rAAVT6 and rAAVT2) baculoviruses.

RESULTS AND DISCUSSION:

We determined optimal production conditions for all the serotypes of rAAV (2, 5 and 6). We showed that regardless of AAV serotype, cell density level had a positive effect on rAAV production. In addition for rAAV2 and rAAV5, Sf9 cell infection at 30°C resulted in a higher titer compared to 27°C. Whereas for rAAVT6 and rAAVT2, MOI of BacRep2 showed a marked effect on the production. The highest titer of rAAV was varying between 1.23E+11 vg/ml for rAAV2 to 9.68E+10 vg/ml for rAAVT6.

Currently, purification of various serotypes of rAAV using affinity and ion exchange chromatography is under investigation.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

OTHER INFORMATION

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EFFECT OF AMINO ACIDS ADDITION IN S2 DROSOPHILA MELANOGASTER CELLS CONTINUOUS CULTURE

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KEY WORDS:
NOVA BIOMEDICAL / PAT / METABOLITE / VIRUS / ANALYSIS

BACKGROUND AND NOVELTY:

Current initiatives within the biopharmaceutical industry to adopt PAT (Process Analytical Technology) approaches in manufacturing, include activities in early process development. With adherent cell lines, online sampling for real-time process monitoring and process adjustment is challenging due to the heterogenity of the cell culture and potential blockages to automated sampling systems caused by microcarriers. In this study, we have tested the BioProfile® FLEX automated sampling and analysis system with OPC (Open Productivity & Connectivity) for continuous feedback control of metabolite concentrations in bioreactors.

EXPERIMENTAL APPROACH:

The first part of the study demonstrated that glutamine, glucose and glutamate consumption was dependent on cell growth phase. In the second part of the study, metabolite concentrations were analysed and controlled in the bioreactor using the OPC enabled BioProfile® FLEX analyser. Sampling was performed via a sterile online sampler, allowing automated nutrient feed control. Once established on non-infected cells, the system was further applied to a model viral (rabies) production process for 14 days. Cell culture experiments were carried out with automated sampling and analysis performed every hour to control and monitor the viral production process.

RESULTS AND DISCUSSION:

This study demonstrates that it is possible to effectively control and adjust, in real-time, the required concentration of metabolites to sustain controlled cell metabolism, as viral infection and production progresses. The BioProfile® FLEX online sampler and analyser, in conjuntion with an OPC enabled bioreactor controller, provided reliable results and improved process control for development activities.

REF-A017

MODIFICATION OF MONOCLONAL ANTIBODY GLYCANS USING GLYCOSYLATION INHIBITORS: EFFECTS ON PRODUCTION, ACTIVITY AND STABILITY

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / INHIBITOR

BACKGROUND AND NOVELTY:
Monoclonal antibodies (Mabs) are now commonly used as biotherapeutic treatments in cancer, autoimmune diseases and other conditions. Commercialization requires high productivity of Mabs in recombinant cell lines, but increasingly product quality with respect to posttranslational modification has been recognized as equally important. N-linked glycosylation of the Fc region (Asn297) in Mabs plays a critical role in their ability to elicit effector functions. Oligosaccharides with low fucosylation allow the Fc region to interact more efficiently with Fc receptors allowing greater effector activation, such as antibody-dependent cell-mediated cytotoxicity (ADCC). Reduction of core fucosylation and an increase in bisecting GlcNAc of Asn297 oligosaccharides has been the focus of several strategies using siRNA and genetic engineering. An alternative method is to use media additives that modify glycans at discrete points during the glycoprotein processing reactions in the Golgi. Mannosidase inhibitors, such as kifunensine, have been shown to generate Mabs with increased effector function.
The objective of this study was to produce mabs with a wide range of glycan microheterogeneity to study the effect of glycosylation on stability and function.

EXPERIMENTAL APPROACH:
We have used several glycoprotein processing inhibitors (kifunensine, swainsonine, castanopermine) to alter glycosylation in recombinant CHO cell lines that produces a chimeric camelid-human Mab (CHO-EG2) and a humanized murine IgG (CHO-DP12).

RESULTS AND DISCUSSION:
Mabs can be efficiently produced in the presence of these inhibitors with little effect on growth of the cells. Glycosylation of the mabs was determined with HILIC (hydrophilic interaction liquid chromatography) analysis showing the modified glycan chains. We have analyzed effects of modifications of the mab glycans on stability, melting temperature, and aggregation using DLS (dynamic light scattering). We have also produced mabs with variable core fucosylation using a new fucosylation inhibitor, a fluorinated analogue of fucose (Rillahan et al 2012), which is incorporated as GDP-F-fucose and inhibits the FUT 8 fucosyltransferase, and are investigating the effects of different levels of fucosylation.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-A018

OPTIMIZATION OF CELLVENTOTM CHO-200 PRODUCTION MEDIA AND FEED PERFORMANCE THROUGH EXPERIMENTAL EVALUATION OF MULTIPLE FED-BATCH PROCESSES

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KEY WORDS:
CHEMICALLY-DEFINED MEDIA / FED-BATCH PROCESS / PROCESS OPTIMIZATION / DESIGN OF EXPERIMENTS / CELL CULTURE PERFORMANCE

BACKGROUND AND NOVELTY:
Successful bio-therapeutic production of monoclonal antibodies and recombinant proteins is highly dependent on the utilization of effective cell culture media that have been optimally designed using quality raw materials, well-defined manufacturing processes, and formulations that are all devised from sound experimentation. Merck Millipore has recently developed and launched Cellvento™ CHO-200, a chemically-defined, animal origin-free production media and companion feed supplement using a rational design of experiments (DOE) approach to optimize their formulations; and qualified raw materials to produce reproducibly consistent dry powder media lots in a cGMP-compliant facility.

EXPERIMENTAL APPROACH:
In order to provide adequate applications support for this media and feed to the upstream biomanufacturing market, we employed a DOE approach to optimize fed-batch media feeding protocols. Specifically, a monoclonal antibody-expressing CHO-S cell line was used to evaluate the impact of media and feeds on cell growth and productivity in small-scale shaker flask and spin tube cultures. The impact of various feed components, volumes, and frequency of administration on overall CHO cell-based monoclonal antibody productivity was investigated. The optimal frequency of feed addition determined in the small-scale fed-batch cultures was confirmed in 3L Cellready bioreactors. In addition to growth curves and titer determinations, JMP statistical DOE software was leveraged to more quantitatively analyze and characterize the contribution of feed components, volume, and frequency on overall CHO cell productivity.

RESULTS AND DISCUSSION:
Our results demonstrated that Cellvento CHO-200 media and feed, when used in an optimized upstream process, yielded superior performance when compared to other commercially-available media and feed offerings, and our experimental findings have been made available to process development scientists interested in optimizing their upstream processes.

DEVELOPMENT OF A DROSOPHILA S2 INSECT-CELL BASED PLACENTAL MALARIA VACCINE PRODUCTION PROCESS

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KEY WORDS:
MALARIA VACCINE / NON-VIRAL INSECT / DROSOPHILA

BACKGROUND AND NOVELTY:
Malaria during pregnancy is the cause of 1500 neonatal deaths a day. Moreover, 40% of all low weight births are caused by pregnancy associated malaria. Researchers at Copenhagen University have identified the VAR2CSA protein as a potential protective recombinant placental malaria vaccine. ExpreS2ion Biotechnologies is responsible for developing the protein production process based on VAR2CSA. This talk will focus on the technology and process development aspects of developing a high-yielding, cost effective, phase I clinical production process based on the ExpreS2 insect cell expression system.

EXPERIMENTAL APPROACH:
This is a novel, non-viral, insect-cell based expression technology applied to the development of a critically needed vaccine. The VAR2CSA protein which the vaccine is based on is hard to express and comparison studies between insect, bacteria and yeast have shown that an insect cell system is the only one leading to a clinically useful immune response. Process optimization is also critically important as the cost of manufacture must be as low as possible to allow the vaccine to be used in the countries where it is most needed.

RESULTS AND DISCUSSION:
S2 cells have proved to be very effective for the production of a broad variety of protein classes, such as viral proteins, toxins, membrane proteins, virus like particles (VLPs), and enzymes. Insect cell-based expression platforms (S2 cells and Baculovirus Expression Vector Systems – BEVS) have now been established as versatile and robust vaccine manufacturing platforms. An efficient manufacturing platform such as the Drosophila S2 cell-based ExpreS2 platform, which is robust and easily scalable, may become critical in enabling the development of some types of vaccines. In addition to the cell line itself; several other components are required to constitute a high performing, recombinant protein production platform.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Charlotte Dyring, MSc PhD, CEO, Founder, ExpreS2ion Biotechnologies ApS, Denmark.

Dr. Dyring (Danish) graduated in Chemical Engineering from the Technical University of Copenhagen (DTU) in 1992 and subsequently received a doctoral degree in combination with an industrial research degree from DTU and the Danish Academy of Technical Sciences, respectively. Dr. Dyring has been working in several scientific positions in the pharmaceutical and the biotechnology industry at Kabi Pharmacia (later Pharmacia & Upjohn) and Pharmexa A/S (Affitech A/S) since 1995. Dr. Dyring has an extensive track record in the field of protein expression in animal cells, mastering a wide array of expression systems, tools and techniques, and she has substantial practical experience with upstream process development according to industry standards, including the process transfer to cGMP manufacturing. Dr. Dyring is a recognized world leading expert of the Drosophila S2 expression technology and was instrumental in the development of the technology to the current level of sophistication and robustness.

ASSESSMENT OF PROCESS PERFORMANCE AND PRODUCT QUALITY IN HIGH PERFORMING FED-BATCH CULTURES

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KEY WORDS:
PRODUCT QUALITY / FED-BATCH / CHO CELLS / IGG PRODUCTION

BACKGROUND AND NOVELTY:
Higher yields, more potent compounds, smaller batch sizes and the cost pressure on R&D budgets push the production of biopharmaceuticals towards single-use bioreactors. Simple cultivation systems can be an attractive platform for the production of e.g. monoclonal antibodies by high cell density fed-batch processes. We have developed a high cell density fed-batch process based on an IgG1 producing CHO cell line and the ActiCHO Media System platform in stirred tank bioreactors as well as in WAVE Bioreactor system. Furthermore, the product quality for key product quality attributes, i.e. glycan distribution, molecular size distribution and charge heterogeneity was analyzed.

EXPERIMENTAL APPROACH:
The development of this high cell density fed-batch process was first established in conventional stainless steel bioreactors up to 100-L cultivation volume. The process yielded maximum viable cell densities in the range of 20 MVC/ml and product titers in the range of 5 g/L. The process was then transferred to a WAVE Bioreactor system and the process performance was evaluated in 5-L, 10-L, 25-L and 100-L scale. The glycan distribution, molecular size distribution and charge heterogeneity was analyzed using LC-MS, SEC and IEX chromatography.

RESULTS AND DISCUSSION:
The process performed comparable across scales and bioreactor systems with respect to viable cell density profiles, viability, metabolite profiles and titers.

In conclusion, WAVE Bioreactors are a functional alternative for the production of monoclonal antibodies in high cell density cell culture processes.

REF-A021

AUTOMATION OF TRANSIENT PROTEIN EXPRESSION IN MAMMALIAN CELLS

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / INHIBITOR

BACKGROUND AND NOVELTY:
The availability of sufficient amounts of proteins, like therapeutic monoclonal antibodies or Fc-fusion proteins, is a prerequisite for their further characterization and optimization within biopharmaceutical research and development. An automated platform for the transient expression of proteins in a mammalian cell line has been established successfully in order to increase throughput and reduce development time. In addition, documentation and traceability is improved.

EXPERIMENTAL APPROACH:
The concept of the automation for the transient expression of proteins is based on an automated platform combining the transfection of a mammalian cell line with a feeding and analysis tool (viable cell density/ml, cell viability, productivity mg/L) and a unique harvesting step of expressed proteins via centrifugation and filtration for downstream purification and further characterization. The cell line used for transient expression is a human embryonic kidney cell line (HEK293-6E) grown in suspension, using 35 ml tube spins with vented lids. The automation concept was developed in cooperation with the company Synchron Lab Automation & Engineering, Netherlands (NL). All main process parts, like transfection, feeding, analysis and harvesting, are programmed as distinct methods and can be selected individually.

RESULTS AND DISCUSSION:
The automated transient expression of proteins is in good correlation to manually performed transient transfections in terms of quality and quantity using HEK293-6E as host cell line. The workstation is able to provide 192 x 35 ml transient transfections in one campaign yielding 250 to 400 mg/L of secreted proteins. In addition to reproducibility and robustness, the incidence of human errors can be reduced. The automation platform contains an integrated data management system connected to our Biologic Data Platform (BDP), thus enabling the documentation and traceability of a large number of different projects at different stages of research and development.

REF-A022

EVALUATION OF THE ADVANCED MICRO-SCALE BIOREACTOR (AMBRTM) AS A HIGHTHROUGHPUT TOOL FOR CULTURE PROCESS DEVELOPMENT

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KEY WORDS:
MICRO-BIOREACTORS / HIGHTHROUGHPUT / CELL CULTURE / PROCESS DEVELOPMENT

BACKGROUND AND NOVELTY:
Bio-pharmaceutical industries face an increasing demand to accelerate process development and reduce its costs. This challenge necessitates highthroughput tools to replace the traditional combination of shake flasks and small-scale stirred tank bioreactor. Here we evaluated and implemented the advanced micro-scale bioreactor (ambrTM) system that has the capabilities for automated sampling, feed addition and pH, dissolved oxygen, gassing, agitation and temperature controls.

EXPERIMENTAL APPROACH:
We evaluated parameters including overall system performance (cell growth and viability, production titer and product quality), reproducibility, comparison to traditional stirred tank bioreactor and tightness of pH dissolved oxygen and temperature control.

RESULTS AND DISCUSSION:
The direct comparison of the ambrTM system with stirred tank bioreactor on the same fed batch process allowed us to fully evaluate the capabilities of this high-throughput system. Based on these results, we are confident to use the ambrTM system to accelerate early stage process development and limit its costs.

DIFFERENTIAL AFFECTS OF LOW GLUCOSE ON THE MACROHETEROGENEITY AND MICROHETEROGENEITY OF GLYCOSYLATION IN CHO-EG2 CAMELID MONOCLONAL ANTIBODIES

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / GLUCOSE CONCENTRATION

BACKGROUND AND NOVELTY:
The demand for high yield recombinant protein production systems has focused industry on culture media and feed strategies that optimize productivity, yet maintain product quality attributes such as glycosylation. Minimizing media components such as glucose and glutamine, reduces the production of lactate and ammonia, but may also affect glycosylation. The first steps in the glycosylation pathway involve the synthesis of lipid-linked oligosaccharides (LLOs) via addition of sugars through nucleotide sugar donors. Glycan macroheterogeneity is introduced by variation in site-specific glycosylation with the transfer of the oligosaccharide to the protein. Further modification of the oligosaccharide can occur through processing reactions, where some sugars are removed and additional sugars added through nucleotide sugar donors. This produces microheterogeneity of the glycan pool. Both macroheterogeneity and microheterogeneity may be affected by the availability of precursors.

EXPERIMENTAL APPROACH:
The objective of this study was to determine how variable concentrations of glucose affect the glycosylation patterns of a camelid monoclonal antibody (Mab) produced in CHO cells and to further evaluate their effect on components of the N-glycosylation pathway, such as nucleotide sugars and LLOs.

RESULTS AND DISCUSSION:
Glucose starvation resulted in a reduction in the amount of full length LLO (GlcNac2Man9Glc3), with a concomitant increase in the production of smaller mannosyl-glycans (GlcNAc2Man2-5). Changes in macroheterogeneity of glycosylation were evident by the appearance of a lower molecular weight protein band identified by mass spectrometry as a non-glycosylated species. Overall N-glycosylation was reduced from 100% to 45% in cells subjected to 24 hours glucose starvation. Glucose deprivation also led to changes in microheterogeneity with a decrease in galactosylation and sialylation.

ENGINEERING CHARACTERISATION OF A SINGLE-USE BIOREACTOR AND THE BIOLOGICAL RESPONSE OF CHO CELLS TO THEIR HYDRODYNAMIC ENVIRONMENT

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KEY WORDS:
SINGLE-USE / PARTICLE IMAGE VELOCIMETRY / CHO CELLS / HYDRODYNAMICS

BACKGROUND AND NOVELTY:
The need to provide cells with reduced hydrodynamic stresses during the upstream processing of mammalian cell cultures, has fostered the production of a number of single-use bioreactors (SUBs) that exhibit novel mixing regimes. With such disparate mixing environments between SUBs currently on the market, the traditional scale-up procedures applied to stirred tank reactors (STRs) are not adequate. The aim of this work is to conduct a fundamental investigation into the hydrodynamics of a single-use bioreactor at laboratory scale to understand its impact upon the growth, metabolic activity and protein productivity of an antibody-producing mammalian cell culture.

EXPERIMENTAL APPROACH:
The SUB to be investigated is the 3L CellReady STR (Merck Millipore) which consists of an upward-pumping marine scoping impeller. This work presents a study characterising the macro-mixing, fluid flow pattern, energy dissipation rates, and shear stresses within the CellReady carried out using 2-dimensional Particle Image Velocimetry (PIV), along with a biological study into the impact of these fluid dynamic characteristics on mammalian cell culture performance and behaviour. The impeller speed and working volume are used to vary the hydrodynamic environment.

RESULTS AND DISCUSSION:
Disparity in cellular growth and viability between the different fluid dynamic environments was not substantial, although a significant reduction in cell specific productivity was found at the most stressful hydrodynamic condition tested. Cells grown at these conditions also displayed net lactate consumption, without a reduction in glucose uptake. A possible reason for these observations is discussed. Given the shifts seen in metabolic behaviour and cell specific productivity, it can be concluded that the fluid dynamic environment will impact upon cellular behaviour. Therefore determining the critical hydrodynamic parameters within the different flow regimes found in SUBs, will enable greater cross-compatibility and scalability across the range of SUBs.

REF-A025

INCLUSION OF MONOCLONAL ANTIBODY CRITICAL QUALITY ATTRIBUTES AS CRITERIA FOR CHEMICALLY-DEFINED CELL CULTURE MEDIA FORMULATION DEVELOPMENT

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KEY WORDS:
CIRITICAL QUALITY ATTRIBUTES / CHEMICALLY-DEFINED MEDIA / CHO / DESIGN OF EXPERIMENTS / GLYCOSYLATION

BACKGROUND AND NOVELTY:
As the biosimilar market emerges and regulation of these therapeutics is considered, the characterization and maintenance of monoclonal antibody critical quality attributes (CQAs) in particular, is drawing scrutiny. Although much effort has focused on understanding the effects of process changes on efficacy-modulating CQAs, cell culture media components can also greatly influence these antibody attributes.

EXPERIMENTAL APPROACH:
Through a series of designed studies we demonstrate the effects of common media components on antibody CQAs. In addition, we demonstrate consistency of these attributes between multiple distinct production lots of CellventoTM CHO-200 dry powder media and feeds, recently developed by Merck Millipore for CHO-S fed-batch processes. Antibody aggregation, charge heterogeneity and Gal(0-2) glycosylation patterns were evaluated, as well as host cell DNA and protein levels. JMP-based data analysis aided the de-coupling of confounding CQA-impacting factors such as viability and day-of-harvest from the effects of media components, including buffer system, on these characteristics.

RESULTS AND DISCUSSION:
The results of this study demonstrate that CQAs are indeed influenced by media formulation modifications and our observations provide the basis for including formulation adjustment as a legitimate area of focus for CQA optimization. In addition to commonly monitored experimental outputs, such as cellular growth and antibody productivity, we include CQAs parameters as criteria for formulation decisions and inputs into predicative formulation modeling. Moreover, we demonstrate the reproducibility of these biotherapeutic characteristics afforded by Merck Millipore's high quality raw materials and manufacturing processes.

REF-A026

POWERFUL EXPRESSION IN CHINESE HAMSTER OVARY CELLS USING BACTERIAL ARTIFICIAL CHROMOSOMES: FINE-TUNING OF PARAMETERS INFLUENCING PRODUCTIVITY

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KEY WORDS:
BIOTECHNOLOGY / CELL CULTURE / CHO / BACTERIAL ARTIFICIAL CHROMOSOME

BACKGROUND AND NOVELTY:
CHO (Chinese Hamster Ovary) cells are the cell line of choice for recombinant protein production and widely used for the production of biopharmaceuticals. Despite the achieved volumetric titers have increased more than 100-fold over the past two decades the establishment of well-producing cell lines remains difficult and is not always successful. Different parameters like the host cell line, the genetic construct, the cell culture medium as well as the applied cultivation strategy are the main factors considered to influence productivity. Recently, Bacterial Artificial Chromosomes (BACs) harbouring the Rosa26 locus showed promising improvements concerning transcriptional efficiency when used as shuttle vector for transgene delivery.

EXPERIMENTAL APPROACH:
In this work a stable and reproducible protocol to generate well-performing cell lines by the use of the BAC expression system in a defined host cell line was established. In a second step the optimal cultivation strategy was evaluated by testing different expression media. The model proteins in this study are IgG1 antibodies and the HIV-1 gp140 envelope protein. The choice of these model proteins provides highly complex molecules with already existing scientific data.

RESULTS AND DISCUSSION:
CHO-K1, -S, -DG44, -DUKX-B11 host cells were compared in different cell culture media. After these preliminary experiments CHO-K1 was chosen for establishment of optimized BAC transfection protocols. Subsequently, stable recombinant cell lines were generated and various process conditions and media compositions are evaluated according to growth rates, maximum cell concentrations and volumetric product titers in batch and fed-batch experiments.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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OTHER INFORMATION
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REF-A027

THE EFFECT OF LOW GLUCOSE AND GLUTAMINE CONCENTRATIONS ON THE GLYCOSYLATION PROFILE OF CAMELID MONOCLONAL ANTIBODY EG2 PRODUCED IN CHO CULTURES

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / GLUCOSE / GLUTAMINE

BACKGROUND AND NOVELTY:
Biological systems are capable of reacting to minimal changes in the environment. These changes can perturb normal cell function and metabolism, which in turn may affect pathways involved in glycosylation. Thus, different strategies in media and feed development have been implemented not only to maximize production rates from available nutrients and minimal byproduct formation, but also to improve product quality. The objective of this study was to determine whether low concentrations of glucose and glutamine had an effect on the glycosylation of a human-camelid monoclonal antibody (CHO-EG2) synthesized during batch culture.

EXPERIMENTAL APPROACH:
For this purpose, cells were cultured for 18 hours in a starvation media and then were split in different shaker flasks with a matrix of various ratios of glucose (3mM to 25mM) and glutamine (0mM to 4mM). Cell growth, substrate consumption, by-product formation and antibody concentration were measured. Glycosylation was analyzed using hydrophillic interaction liquid chromatography (HILIC).

RESULTS AND DISCUSSION:
The chosen range of glutamine did not have an effect on cell growth. However, in the absence of glutamine and at reduced glucose concentrations there was a decrease in cell growth, which in turn affected product formation. Using HILIC we showed that Mabs produced under glutamine deprivation combined with low levels of glucose contained a higher percentage of truncated oligosaccharides, with a reduced sialylation compared to the Mabs produced under normal conditions (4mM Gln, 25mM Glc). However, the galactosylation index was not changed. Isolated Mabs produced under low glucose conditions produced two bands by SDS-PAGE chromatography. The appearance of a lower molecular weight band was confirmed to correspond to a non-glycosylated Mab by mass spectrometry analysis, indicating a change in the macroheterogeneity.

REF-A028

ENHANCED INSIGHT INTO THE CAP AND CAP-T EXPRESSION SYSTEMS AND THEIR PROPERTIES

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KEY WORDS:
CAP / CAP-T CELLS / RECOMBINANT PROTEIN PRODUCTION

BACKGROUND AND NOVELTY:
The CAP cell line has recently been established from primary human amniocytes by immortalization as a novel cell line for recombinant protein production. Complementary to this one clone of the cell line was stably transfected to express the SV40 Large T Antigen gene. This gave rise to the CAP-T cell line claimed to enable enhanced transient gene expression in combination with expression vectors carrying the SV40 Origin of Replication. We tested both the CAP and the CAP-T cell lines in the context of protein production for research purposes and explored a variety of transfection process parameters aiming at increased protein titers.

EXPERIMENTAL APPROACH:
For transient transfection of CAP-T cells we investigated the influence of medium, transfection reagent, starting cell density and addition of known expression enhancers to the growth medium. After developing a robust protocol for polyethyleneimine (PEI)-mediated transient transfection at high cell density, this protocol has successfully been applied to the generation of antigens and selection of antibody candidates on small to medium scale. In addition we evaluated the potential of CAP cells by establishing probably episomally stable and stably integrated, MTX amplified cell pools by nucleofection and selection.

RESULTS AND DISCUSSION:
Our comparative results which will be presented show that the CAP/CAP-T cell system is an attractive alternative to the repertoire of existing host cell lines such as HEK293 and CHO cells for transient and stable recombinant protein production in research.

REF-A029

ANTIVIRAL ACTIVITY AGAINST INFLUENZA, MEASLES AND PICORNAVIRUS OBSERVED IN THE HEMOLYMPH OF PODALIA SP (LEPIDOPTERA: MEGALOPYGIDAE)

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KEY WORDS:
ANTIVIRAL / LEPIDOPTERA / INFLUENZA / MEASLES / BIOPROSPECTION

BACKGROUND AND NOVELTY:
The control of human viruses is of high interest in human and animal health. Despite the frequent appearance of drug resistant viruses, the development of new antiviral agents is needed to support medicine. Several works have demonstrated the presence of bioactive peptides and their potential use as therapeutic agents in insect hemolymph. However, relatively little data are available on molecules from insects with antiviral activities.

EXPERIMENTAL APPROACH:
In this study, the effects of supplementation of infected culture with hemolymph from larvae of Podalia sp (Lepidoptera: Megalopygidae) were investigated. Cytotoxicity and genotoxicity was evaluated, and no adverse effects were observed in culture after hemolymph addition (up to 5%). The effect of hemolymph on virus growth was measured on confluent monolayers of infected cells with measles virus, influenza virus (H1N1) (enveloped virus) and picornavirus (non enveloped virus). The cultures were observed daily for evidence of cytopathic effect. The analyses of the viral titer demonstrated that the addition of 1% of Megalopygidae hemolymph decreased significantly ($p=0.002$) the virus titer. The antiviral protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel filtration column system (Superdex 75) and further fractionated using a Resource-Q ion exchange column system.

RESULTS AND DISCUSSION:
Experiments with the purified protein led to a 32-fold reduction in influenza virus production, 64-fold reduction in measles virus production and a 256-fold reduction in picornavirus production. Heating and freezing seem to have no influence over its antiviral activity. The protein does not display virucidal activity and does not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action where the protein may act as a constitutive agent that affects the innate antiviral immune response.

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REF-A030

OPTIMIZED FERMENTATION CONDITIONS FOR IMPROVED ANTIBODY YIELD IN HYBRIDOMA CELLS

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KEY WORDS:
HYBRIDOMA CELLS / DESIGN OF EXPERIMENT / FERMENTATION / HUMAN INSULIN-LIKE GROWTH FACTOR / MEDIA OPTIMIZATION

BACKGROUND AND NOVELTY:
Ever since new suspension cell lines as adapted HEK293, CHO or Per.C6 have captured the biopharmaceutical production market, traditionally antibody producing cells like Hybridoma cells sank into oblivion. However, they are still of particular interest in academic and industrial diagnostic research for fast and sufficient antibody production needed as proof of concept, for toxicology and in vivo studies. Although, Hybridoma cultivation in fetal bovine serum (FBS) containing animal derived ingredients, like contaminating IgG, is undesirable and leads to difficulties in purification. When reducing the serum to a minimum other key components of the FBS have to be replaced. Therefore, human insulin-like growth factor (IGF) and the surfactant Pluronic F68 were supplemented to improve overall cell performance and to reduce shear forces during shaking respectively employing Design of Experiment (DoE).

EXPERIMENTAL APPROACH:
DoE was used to lower FBS concentration, combined with supplementation of IGF and Pluronic F68. Cells were cultivated for five days in shaker flask. Cell concentration and viability were quantified every day and were defined as response factors for DoE analysis. The response factors were used from exponential growth phase and analyzed with the DoE software Modde, Umetrics. Cultures grew with optimized conditions were used as inoculum for subsequent bioreactor fermentations.

RESULTS AND DISCUSSION:
Reduction of FBS without supplementation resulted in decreased viability and cell concentration. However, FBS can be decreased from 10% to 6% by adding 100 µg/L human IGF and 0.2 g/L Pluronic F68. Compared to the original basal medium an improvement in cell growth and viability was achieved. For entirely serum-free Hybridoma culture further critical ingredients like transferrin and albumin have to be replaced. However, serum-free media leads to higher production costs and can result in a reduction in antibody yield. Nevertheless DoE is a powerful and effective tool saving time in process optimization.

EFFICIENT CLONING OF SINGLE CHO-S CELLS USING A NOVEL ANIMAL COMPONENT-FREE CULTURE MEDIUM SUPPLEMENT

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KEY WORDS:
CHO CELLS / BIOPROCESSING / MONOCLONAL CELL LINES / CLONING EFFICIENCY / MONOCLONAL ANTIBODIES

BACKGROUND AND NOVELTY:
Over the past decade, the number of biotherapeutic drugs produced in Chinese hamster ovary (CHO) cells has increased dramatically. To achieve consistently high expression of a protein product, monoclonal cultures of transfected CHO cells are generated by single-cell cloning. Efficient expansion of single CHO cells typically requires the use of medium containing fetal bovine serum (FBS) or alternatively, use of conditioned medium or co-culture with feeder cells. However, these systems are not defined, and batch variation and risk of contamination from adventitious agents make use of FBS undesirable. To address these issues, we have developed a defined, animal component-free (ACF) culture supplement containing only recombinant proteins and synthetic components that significantly increases CHO cell cloning efficiency when added to protein-free media.

EXPERIMENTAL APPROACH:
The cloning efficiency of CHO-S cells plated at limiting dilution in 96-well plates (average 1 cell/well) was compared in different commercially available, protein-free culture media supplemented with L-glutamine with or without ACF supplement. Cells grown in Dulbecco's Modified Eagle Medium (DMEM) plus 10% FBS were used as a positive control. Cultures were incubated for 10-14 days and screened to identify wells containing >100 cells/well.

RESULTS AND DISCUSSION:
Growth of CHO-S cells in DMEM plus 10% FBS resulted in ~50% of wells containing >100 cells. No significant cell growth was observed in any of the tested media in the absence of serum. The addition of ACF CHO supplement rescued cell proliferation in all four media without serum, resulting in cloning efficiencies that ranged from 50-100% of that observed in DMEM plus 10% FBS. These results show that a new ACF CHO supplement supports high cloning efficiencies for single CHO-S cells cultured in commonly used, commercially available, protein-free media. This completely defined ACF culture system should increase the reproducibility and productivity of biological drug manufacturing.

OBTAINING OF A PROPRIETARY CHO CELL LINE, EVALUATION OF GLUTAMINE SYNTHETASE GENE KNOCK-OUT AND PRODUCTION OF ANTI-RABIES MAB USING AUTOMATED ANALYSIS AND AMBR MINI BIOREACTORS

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KEY WORDS:
CHO CELL LINE / AUTOMATION / SCALE-UP / AMBR / KNOCK-OUT

BACKGROUND AND NOVELTY:
Sanofi developed an in-house expression platform incorporating a fully documented CHO cell. First a CHO bank was thawed in a serum free medium then the cell line was adapted to grow individually in suspension in a chemically defined medium. The Premaster Cell Bank generated was extensively tested against contaminating agents.

EXPERIMENTAL APPROACH:
The selection of producers was based on the glutamine synthetase enzyme produced at a basal level by the CHO cell. This basal expression level was inhibited by the addition of Methionine Sulfo Ximine (MSX) in the selection medium. The gene of this essential enzyme was brought with the gene of interest in order to select the highest producers. However, this chemical selection may have an impact on cell growth and is not absolute.

The knock-out (KO) of the glutamin synthetase gene (GS) was performed on the Sanofi cell line. In the absence of an endogenous GS gene, no selection escape is possible and as MSX is not required, cell growth can be improved. In this experiment, we evaluated the benefit of KO GS clones combined with the need to produce SO57 (an anti-rabies mAb).

After transfection of both the parental and KO GS CHO cell lines, a selection was performed on 96 well plates with an automated Homogeneous Time Resolved Fluorescence HTRF assay. The highest producers were amplified and banked. In order to choose the cell line and the process for the production of the mAb, both were jointly tested in an Ambr mini-bioreactor automate.

The knocking out of the GS gene allowed enrichment with high producers of the transfected pool. The evaluation in the Ambr platform led to the selection of an improved couple cell line / process in one step.

RESULTS AND DISCUSSION:
These results were applied on a 30 L single use bioreactor resulting in a harvesting of 30 g of antibody. The new expression platform coupled with the automation of the analytical and process tools reduced the time from transfection to batch production by up to 3 months.

REF-A033

PROCESS DEVELOPMENT STRATEGIES TO ENABLE LARGE SCALE EXPANSION OF MESENCHYMAL STEM CELLS FOR CELLULAR THERAPY

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KEY WORDS:
MESENCHYMAL STEM CELLS / CELL THERPAY / PROCESS DEVELOPMENT / MICROCARRIER / SERUM-FREE MEDIA

BACKGROUND AND NOVELTY:
The potential demand for clinical and commercial scale human mesenchymal stem cells (MSC) for cellular therapies requires a large-scale and well characterized culture system for MSC production. Currently, the majority of processes to produce MSCs rely on 2-dimensional, planar technologies that are expensive, labor intensive, and limited in scale potential. Strategies for process development have been used successfully in other industrial therapeutic markets such as the monoclonal antibody and vaccine industries to increase product yield. Many of these approaches such as Design of Experiment-based optimization (DOE) strategies can be used to develop cell culture media, reagents, and scale-up methods that may make cost effective and efficient manufacturing processes possible for cell therapy-relevant cells.

EXPERIMENTAL APPROACH:
Here we report the results of DOE-based optimization studies to develop a microcarrier-based expansion system for human MSCs. Spinner flask studies demonstrated the ability of a xeno-free system to support expansion of MSC from bone marrow (BM MSC) and adipose tissue (ADSC) while maintaining the expected phenotype and differentiation potential. After 14 days of culture, BM MSC reached a maximum cell density of 200,000 cells/ml (fold-increase of 18) while ADSC expanded to 140,000 cells/ml (fold-increase of 14). Medium and process optimization strategies and the incorporation of fed-batch and perfusion approaches were used to increase the efficiency of the system. Human MSCs were expanded to a cell density of greater than 500,000 cells/ml in DASGIP bench-top bioreactors. The cells maintained tri-lineage differentiation potential and retained the MSC immunophenotypic profile.

RESULTS AND DISCUSSION:
This work demonstrates the ability of a serum-free and xeno-free medium to support large-scale expansion of human MSC. This system can produce large numbers of high quality MSC, representing an efficient alternative to the traditional cell expansion protocol for clinical-scale manufacture of MSC.

REF-A034

SCALABILITY STUDIES OF THE UPSTREAM MANUFACTURING PROCESS DURING THE DEVELOPMENT OF ANTIBODY MIXTURES

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KEY WORDS:
ANTIBODY / MIXTURES / CELL CULTURE / SCALABILITY

BACKGROUND AND NOVELTY:
The synergistic effects of combinations of antibodies reported in particular for cancer treatment demonstrate that antibody mixtures represent a promising new class of therapeutics. Therefore, recombinant antibody combinations or mixtures are receiving more and more attention as a strategy to improve therapeutic efficacy.

Symphogen is using the SympressTM platform process for single-batch manufacture of antibody mixtures. Using this manufacturing platform, it is possible to produce several antibodies in a mixture with suitable quality characteristics and high batch-to-batch consistency.

EXPERIMENTAL APPROACH:
During the development of antibody mixture products, it is important to have scalable models of the upstream process that can be used as a tool for efficient early development as well as be used as an important predictive tool for later-stage investigations of the process.

RESULTS AND DISCUSSION:
We here present data from several upstream models that we have developed for manufacturing of antibody mixtures ranging from deep-well plates run in a robotized format through micro-bioreactors to traditional shakers and bioreactors. The combination of high-throughput analytical tools and upstream models at several scales has allowed us to systematically develop and refine the Sympress platform technology so it delivers robust high-quality antibody mixtures.

STEM CELLS CULTURE ON MICROCARRIERS INSIDE SHAKE FLASKS: 2 - KINETIC STUDY AND COMPARISON WITH CULTURE IN SPINNER FLASKS

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KEY WORDS:
STEM CELLS / SHAKING FLASK / KINETICS

BACKGROUND AND NOVELTY:
Mesenchymal stem cells (MSC) are promising tools for tissue engineering and will be required in sufficient amounts. However, the choice of bioreactor technology as well as aeration and agitation conditions is still challenging for their mass expansion. Spinner flasks were already proved to be suitable for MSC culture on microcarriers. While orbitally shaken bioreactors were used for continuous cell lines and present some advantages such as reduced cost, sufficient oxygen transfer capacity and good scale-up ability, they have not been yet validated for MSCs culture. In this work, kinetics of MSCs cultures were studied in orbitally shaken flasks and compared with results obtained in spinner flasks.

EXPERIMENTAL APPROACH:
Porcine MSC were seeded at 0.3 x 10^5 cells/mL on 1.2 g/L Cytodex 1 microcarriers in -MEM supplemented with 10 % SVF and FGF2. Shake flasks (250 and 500 mL) equipped with O2 and pH optical sensors (Presens), were placed into an incubator (Kühner). Concentration of cells, glucose, glutamine, lactate and ammonium ions was daily determined. Cell multipotency was assayed by using differentiation kits. Additional cultures were performed in 250 mL spinner flasks. Two working volumes were used for each culture system and medium was renewed every 1 or 2 days.

RESULTS AND DISCUSSION:
Whatever operating parameters, MSC growth, nutrient consumption and metabolite production were observed to be very similar in shake and spinner flasks, leading to a 40-fold increase of cell density after 10 days. Yet, cell aggregates formed quicker and reached a larger size in spinner flask as revealed by microscopic observations. Despite smaller mean power dissipations estimated in spinner flask, shake flask offered a non-turbulent flow and a more homogeneous distribution of hydromechanical stresses, which seemed favourable to MSC growth. Moreover, the oxygen profiles observed in shake flasks revealed that low pO2 conditions could be obtained by a judicious choice of the shaking frequency and medium volume.

STEM CELLS CULTURE ON MICROCARRIERS IN SHAKING FLASKS : 1 - EXPERIMENTAL DETERMINATION OF MINIMAL SHAKING FREQUENCY AND DIMENSIONAL ANALYSIS TO GET MICROCARRIERS INTO SUSPENSION

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KEY WORDS:
SHAKE FLASK / ORBITAL SHAKING / MICROCARRIER / DIMENSIONNAL ANALYSIS / MAMMALIAN CELL CULTURE

BACKGROUND AND NOVELTY:
Shake flasks are widely used as lab-scale bioreactors for culturing mammalian cells, these systems being able to support culture processes in terms of mixing and hydro-mechanical forces characteristics [1, 2, 3]. For adherent cell lines cultured on microcarriers, such as mesenchymal stem cells, defining the impact of the shaking frequency on the process has to include the actual setting up of the microcarriers into suspension. While correlations are available in literature to predict the minimum agitation speed, Nc (1/s), necessary to get microcarriers in suspension in mechanically agitated bioreactors [4], such correlation does not yet exist for orbital shaking culture systems. Our study offers to fill this gap by using a rigorous dimensional analysis of physics.

EXPERIMENTAL APPROACH:
Experimental measurements of Nc have been made by observing shake flasks on an orbital shaking platform (Kuhner) and visually checking the suspension state of stained Cytodex 1 carriers within the flasks. About 200 various experimental conditions, were tested: shake flask diameter, d, flask filling volume, orbital shaking diameter, d0, liquid viscosity, volume fraction of particles, particle diameter, and particle mass density. The values of these parameters were based on classical adherent-cell culture parameters.

RESULTS AND DISCUSSION:
Regarding the dimensional analysis of the process, two correlations were established to evaluate a critical Froude number, used to determine the resistance of submerged carriers moving through liquid medium. The first one directly used Nc, while the second one was based on an intermediate variable: the critical shaking velocity Vc (m/s), depending of Nc, d0 and d values. The identification of model parameters leads to a final average error less than 5 %. Thus, our results allowed to establish robust correlations that characterize the influence of diverse operating parameters on the minimal shaking frequency (Nc) to get microcarriers into suspension.

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REF-A037

BIOLOGICS DATA PLATFORM FOR TAILORED SUPPORT OF AUTOMATION IN TRANSIENT PROTEIN EXPRESSION AND CELL LINE DEVELOPMENT

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KEY WORDS:
BIOLOGICS DATA PLATFORM / TRANSIENT TRANSFECTION / CELL LINE DEVELOPMENT / AUTOMATION WORKSTATIONS / ENTERPRISE-IT SOLUTION

BACKGROUND AND NOVELTY:
The biologics drug R&D process in pharmaceutical and biotech companies is characterized by division of labor across sites and high-throughput approaches. The integrated management of data from molecules, clones, materials and analytical experiments is a key challenge in this context. We have successfully implemented the Biologics Data Platform (BDP), a novel enterprise-IT solution based on Genedata Biologics software, for tailored support of our screening and protein production processes. Here, we describe the integration of BDP with our automation workstations in transient transfection for mg-scale protein expression and cell line development.

EXPERIMENTAL APPROACH:
We implemented an automated workstation for parallelized transient transfection, feeding, sampling and harvesting of 35 ml-spin tube cultures. We implemented vector batch and expression batch registration processes in BDP to guide the automated workstation and to receive analytics data from the workstation.

Additionally, we implemented an automated cell line development workstation for seeding, selection, incubation, passaging, analyzing, and cryo-conservation of cells. Also here we implemented functionalities in BDP to support the cell line development process.

RESULTS AND DISCUSSION:
We demonstrate the advantages of comprehensive management in one IT system of transient expression batches, cell line clones and fed-batch experiments together with molecule information such as primary sequences and experimental data. The system facilitates correlation analyses which will be discussed with examples.

REF-A038

IMPACT OF MIRNAS ON THE CHO PROTEOME

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KEY WORDS:
CHO / MIRNA / LABEL-FREE LC-MS / BIOINFORMATICS / MIR-7

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are small non-coding RNAs (~22 nucleotides in length) that can post-transcriptionally regulate gene expression through inhibition of protein translation or degradation of target mRNAs. MiRNAs play critical roles in the regulation of biological processes such as growth, apoptosis, productivity and secretion thus representing a potential route toward enhancing desirable characteristics of mammalian cells for biopharmaceutical production. Relatively few studies to date explore the potential of miRNAs as cell line engineering tools for bioprocessing. This is vitally important if they are to be exploited for cell line engineering for process improvements.

EXPERIMENTAL APPROACH:
We have previously found that miR-7 over-expression significantly inhibits the growth of recombinant Chinese hamster ovary (CHO) cells without impacting cellular viability, with an associated increase in normalised productivity. In this study we have carried out a quantitative label-free Liquid Chromatography – Mass Spectrometry (LC-MS) proteomic profiling study of proteins exhibiting altered levels following over-expression of miR-7 to gain insights into the potential mechanisms involved in the observed phenotype.

RESULTS AND DISCUSSION:
In total 93 proteins showing decreased levels and 74 proteins with increased levels following over-expression of miR-7 were found. Pathway analysis suggests that proteins involved in protein translation (e.g. ribosomal proteins), RNA and DNA processing (including histones) are enriched in the list showing decreased expression. Protein folding and secretion proteins were found to be up-regulated following miR-7 over-expression. Bioinformatic analysis with miRWalk (combines the output of 6 selected miRNA target prediction algorithms) was used to evaluate potential direct targets of miR-7. Two genes, stathmin and catalase, overlapped in a number of the predictive target databases for mouse and rat, and are likely to be possible direct targets of miR-7 in CHO cells

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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FACILITATING MULTISITE BIOPROCESS TRANSFER: MULTI-INSTRUMENT AND MULTI-PLATFORM COMPARABILITY AND LONG-TERM NOVA BIOMEDICAL'S BIOPROFILE® CHEMISTRY AND GAS ANALYZERS

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KEY WORDS:
PROCESS TRANSFER / COMPARABILITY / ANALYZER / NOVA BIOMEDICAL / PROCESS MONITORING

BACKGROUND AND NOVELTY:
The Biopharmaceutical industry has grown exponentially and more companies are now operating in a global market with sites thousands of miles apart. The need is now even greater for robust bioprocess monitoring solutions that can provide consistent instrument-to-instrument results. The seamless transfer of information across multiple sites relies heavily on the comparability of process data from various technologies, ensuring effective monitoring and control of critical process parameters.

EXPERIMENTAL APPROACH:
This study provides data supporting comparability of the BioProfile® (Nova Biomedical, Waltham, MA) chemistry and gas analyzers across several development and manufacturing sites in the United States. In addition, the long-term performance stability of the BioProfile systems was also tested. Five BioProfile FLEX and four BioProfile 100 Plus analyzers were used to determine linearity, precision, accuracy, and instrument-to-instrument comparability. The age of the instruments used for this study ranged from new to over 8 years old, with several hundred samples to over 20,000 samples run on a given analyzer.

RESULTS AND DISCUSSION:
The results of this study show a high level of comparability between the BioProfile analyzers. In addition, comparability was also demonstrated between both the new and aged analyzers, providing evidence of the long-term robustness and the quality of data that can be generated from the BioProfile analyzers. Nova Biomedical's BioProfile analyzers provide the tools to facilitate multisite bioprocess transfer in the Biopharmaceutical industry.

ON-LINE MONITORING OF ADHERENT CELLS CULTIVATED ON MICROCARRIERS IN PERFUSION REACTOR

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KEY WORDS:
MULTI-FREQUENCY PERMITTIVITY / PERFUSION BIOREACTOR / MICROCARRIERS / VERO CELLS / BIOPROCESS MONITORING

BACKGROUND AND NOVELTY:
Animal cell density is a critical parameter to on-line monitor culture processes. Some industrial processes currently cultivate anchorage-dependent cells on microcarriers (MCs), such as Vero cells for vaccine production. Expansion of stem cells could also require the use of microbeads. Perfusion reactors allow to reach high cell densities which may result in carrier saturation. So, monitoring high densities of viable anchorage-dependent cells by using a capacitance probe remains challenging. Our aim was to deeply consider the effect of Vero cell confluency on MCs on the on-line monitoring of high cell densities by permittivity measurements.

EXPERIMENTAL APPROACH:
Cells were cultivated in a serum-free medium on spherical Cytodex 1 microcarriers inside a 2 L perfusion reactor using a settling retention system and a flow rate of 0.5 L/d. Various microcarrier concentrations (1.5, 3 and 6 g/L) were used. Off-line cell quantification was performed by Crystal violet method, while cell diameter and viability were assessed by Vi-CELLTM system. On-line permittivity was measured by using Fogale Biomass system® over a frequency range of 0.3 to 10 MHz.

RESULTS AND DISCUSSION:
Depending on the operating conditions, various maximal cell concentrations between 2 and 4.5 x 10E6 cells/mL (100 to 500 cells/MC) were reached and maintained during more than 8 days. Results showed a linear correlation between on-line permittivities and off-line volumetric cell densities, even for the highest volumetric cell concentrations. This correlation was not affected by MCs concentration but was no longer linear when cell number per MC exceeded 150. This behavior was attributed to the diameter decrease of Vero cells on microcarrier surface, resulting from high cell density in perfusion reactor. For the first time, our results demonstrate that permittivity sensor can be considered as a reliable tool to monitor high adherent cell densities, provided they do not exceed cell confluency on adhesion surface, and to detect cell confluency occurrence.

REF-A041

BENCHMARKING OF COMMERCIALLY AVAILABLE CHO CELL CULTURE MEDIA FOR ANTIBODY PRODUCTION

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KEY WORDS:
ANTIBODY, MEDIUM, CHO CELLS, RECOMBINANT PROTEIN PRODUCTION

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO) cells have become the preferred expression system for recombinant proteins. A key factor for a high yield process is the cultivation medium. We investigated 8 commercially available CHO cell culture media to examine their impact on cell growth, recombinant protein production and cell metabolism.

EXPERIMENTAL APPROACH:
A recombinant CHO DG44 cell line expressing a human IgG1 antibody was cultivated in shake flasks to compare the following media:
- ActiCHO P (GE Healthcare)
- CD Opti-CHOTM (Life Technologies)
- CD Opti-CHOTM (Life Technologies)
- CD Forti-CHOTM (Life Technologies)
- Ex-CellR CD CHO (Sigma Aldrich)
- ProCHOTM 5 (Lonza)
- BalanCDTM CHO Growth A (Irvine Scientific)
- CellventoTM CHO-100 (EMD Millipore)

For ActiCHO two feed strategies, initial spiking or daily addition of supplements were investigated. Also in BalanCD medium one culture was run as fedbatch adding Feed 1 on days 1, 3, 5.

The cultures were sampled daily to determine cell growth, viability, IgG and metabolite concentrations including amino acid analysis.

RESULTS AND DISCUSSION:
Selection of an appropriate medium was crucial for high antibody production. The cell concentration was increased up to threefold from 2.59x106 c/mL to 7.73x106 c/mL. Similarly the final product concentration rose from 384 mg/L to 876 mg/L.

An adequate feeding strategy further boosted cell concentrations to 1.99x107 c/mL and titers to >5 g/L. The specific productivities (qP) were similar for all non-fed cultures. There the qP gradually declined from 50-70 pg/(c*d) (pcd) to 8 pcd during the cultivation. In feed-spiked ActiCHO P a similar trend was observed but the qP was always 30-80% higher. Contrastingly, a daily feed stabilized the qP at 50 pcd for several days.

Spent medium analysis revealed that high concentrations as well as a balanced proportion of amino acids were important for a good productivity. Besides glutamine and glutamate, critical amino acids for this cell line turned out to be tyrosine, asparagine and serine.

REF-A042

WHEN QUALITY COMES FIRST- CHALLENGES FOR MAMMALIAN CELL LINE DEVELOPMENT OF “DIFFICULT TO EXPRESS” PROTEINS

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KEY WORDS:
NGNA / AGGREGRATES / CELL LINE DEVELOPMENT / NOVEL ASSAY / PRODUCT QUALITY

BACKGROUND AND NOVELTY:
Productivity has always been a primary criterion for clone selection during cell line development since it is directly associated with the cost of goods manufactured. While high productivity is desired, product quality issues, such as immunogenic factors, sometimes become unpleasant surprises at the later stage of the development program. Product aggregation and the presence of N-glycolyneuraminic acid (NGNA or Neu5Gc) have been linked to increased product immunogenicity. Addressing these risk factors early can potentially reduce the overall immunogenicity risk profile of the product.

EXPERIMENTAL APPROACH:
Production of BMN-X molecule in CHOK1 cells results in two activity peaks separated by anion exchange chromatography. Size exclusion chromatography detected various HMW forms in the later peak. A simple charge heterogeneity method for the quantification of the HMW forms can be used to rapidly screen clones, production conditions and new designs which favor the formation of the correct form of BMN-X.

CHO cells produce glycosylation patterns that are close to human, however, these cells do express NGNA and this expression seems to be cell type and clone specific. We implemented a 96-well plate based ELISA assay using an antibody specific to NGNA to identify and eliminate clones with high levels of Neu5Gc glycosylation early in development.

RESULTS AND DISCUSSION:
In conclusion, the ability to identify and eliminate clones with high levels of NGNA or a high percentage of HMW forms early in development allowed us to focus only on clones with better quality attributes. Traditionally, these assays were performed with purified protein samples less conducive to high throughput screening methods. Implementing these new screening assays enabled us to assess preliminary product quality with µL to mL quantity of cell culture harvest fluid. In addition, we discuss the impact of cell culture media and culture process on the levels of HMW material and NGNA, and report strategies aiming to reduce these undesired forms.

RAMAN SPECTROSCOPY AS A TOOL FOR THE MONITORING AND CONTROL OF MAMMALIAN CELL CULTURE BIOREACTORS

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KEY WORDS:
RAMAN SPECTROSCOPY / GLUCOSE CONTROL / MODEL PREDICTIVE CONTROL / PAT

BACKGROUND AND NOVELTY:
The philosophy behind the FDA's process analytical technology initiative and the move from quality by inspection to quality by design (QbD) is that identifying, monitoring and controlling the critical process parameters (CPPs) positively impacts the critical quality attributes (CQAs) of a product as a result of better process understanding and control, earlier fault detection and continuous process improvementTo achieve this higher degree of process control, development of online process monitoring is essential. Raman spectroscopy has great potential as an analytical tool for bioprocesses: it is non-invasive, non-destructive, does not require sampling, can quantify multiple analytes simultaneously and provide continuous real-time measurements.

EXPERIMENTAL APPROACH:
Raman spectroscopy was used for the in situ real-time quantitative determination of glucose, glutamine, lactate, ammonia, glutamate, total cell density (TCD) and viable cell density (VCD) in a CHO cell fed-batch process. Chemometric data analysis was used to correlate the spectral data with off-line reference methods. The effect of the number of calibration samples and variation within the calibration sample set was investigated as well as the transferability of the calibration models from a 3 L reactor to 15 L system.

RESULTS AND DISCUSSION:
Calibration models were successfully built using data acquired over the course of three bioprocess runs. The standard error of prediction was 1.82 mM for glucose, 0.44 mM for glutamine, 0.22 mM for glutamate, 0.13 mM for ammonia, 9.77 mM for lactate and 0.43 x 106 cells/ml for TCD. The Raman-determined glucose concentration was used as part of a closed-loop feedback control strategy which maintained glucose at a setpoint. A form of advanced control called model predictive control (MPC) was implemented to successfully maintain glucose at a fixed concentration of 11 mM. A 1.5 fold increase in maximum viable cell density was observed when the MPC-controlled bioprocess was compared with a bolus fed-batch process.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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ENGINEERING CHARACTERISATION OF A ROCKED BAG BIOREACTOR TO EVALUATE KEY EFFECTORS OF SUCCESSFUL MAMMALIAN CELL CULTURE

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KEY WORDS:
ROCKED BAGS / MAMMALIAN CELL CULTURE / ENGINEERING CHARACTERISATION / OXYGEN TRANSFER / CO2 STRIPPING

BACKGROUND AND NOVELTY:
Engineering characterisation is essential for efficient and knowledge-led process development in biomanufacturing. Despite diverse applications of rocked bag bioreactors, there is currently little understanding of the fundamental determinants of fluid mixing and mass transfer, which are important for mammalian cell culture.

EXPERIMENTAL APPROACH:
In this work, a flexible single-use rocked bag bioreactor system has been fully evaluated in terms of volumetric oxygen mass transfer coefficient (kLa), CO2 stripping rate and liquid phase mixing time at 10L and 20L scale. Five inputs were identified as potentially affecting gas transfer and mixing characteristics: rocking rate, rocking angle, fill volume, rocking acceleration and air flow rate. Using these findings, industrially relevant fed-batch GS-CHO cell cultures were then conducted to demonstrate the effects of these parameters on cell growth, productivity and metabolite profile.

RESULTS AND DISCUSSION:
It was found that oxygen transfer could be an issue for mammalian cell culture at the cell densities reached in industrial fed-batch processes. Within sensible operating ranges for cell culture, the oxygen mass transfer coefficient, kLa, was most sensitive to rocking rate and fill volume (5-fold higher at 25rpm compared to 15rpm). Bubble formation and the presence of a dispersed gas phase was observed at moderate rocking rates and was prevalent at high rocking rate or low fill volume. In terms of scale-up, kLa did not change significantly with a doubling in scale. In contrast, the liquid phase mixing time, approximately doubled. CO2 stripping was largely determined by air flow rate at both 10 and 20L scales but was proportionately slower at 20L scale. Cell culture at 10L scale confirms the importance of kLa evaluation with regards to cell growth and antibody production and CO2 stripping in terms of process control, but these results suggest that the relative importance of different engineering parameters will alter upon scale-up.

REF-A045

MULTIDIMENSION CULTIVATION ANALYSIS BY STANDARD AND OMICS METHODS FOR OPTIMIZATION OF THERAPEUTICS PRODUCTION

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KEY WORDS:
CHO / CELL CULTURE MEDIUM / TRANSCRIPTOMICS / METABOLOMICS

BACKGROUND AND NOVELTY:
During the last decades Chinese Hamster Ovary (CHO) cells have been extensively used for research and biotechnological applications. About 40% of newly approved glycosylated protein pharmaceuticals are produced in these cells today [1]. Despite the increasing relevance of CHO cells for biopharmaceutical production little is known about effects of intracellular processes on productivity and product quality.

EXPERIMENTAL APPROACH:
In the last years insulin was more and more replaced by IGF in cell culture media. To compare the intracellular effects of these two supplements an antibody producing CHO cell line was cultivated in batch mode using insulin, IGF-1 or no growth factor. Subsequently, different omics-techniques were applied to analyze medium and cell samples. Metabolome and glycan analysis was performed using a HILIC-ESI-MS and a HPAEC-PAD method, respectively. Furthermore, an in house developed customized cDNA microarray with 41,304 probes based on sequence data from different CHO cell lines was applied for transcriptome analysis.

RESULTS AND DISCUSSION:
Cultivation data illustrated that maximal cell density was higher in cultivations with insulin and IGF-1 compared to those without growth factor. Additionally, glucose consumption and lactate production was slightly higher in cultivations with these supplements. In contrast to that product quantity and product quality was equal in all cultivations. The most abundant glycoforms were G0F and G1F with about 50% and 40%, respectively. Transcriptome data showed that IGF supplementation result in the highest significant transcription change, e. g. on day three of cultivation 3,187 probes in IGF samples and 1,214 probes in insulin samples indicated different transcription levels.

Data on cell growth and productivity as well as omics results were brought together to achieve a deeper insight into cellular processes and their influence on productivity and product quality.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-A046

QUALITY BY DESIGN IN RAW MATERIALS TESTING: CONSIDERATIONS, STRATEGIES AND EXPERIENCE OF A TESTING LABORATORY

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KEY WORDS:
RAW MATERIALS / QBD / QUALITY BY DESIGN / TESTING

BACKGROUND AND NOVELTY:
Testing of raw materials is an essential step in the production cycle of biological therapeutics and vaccines. The implementation of Quality by Design (ICH Q8) in manufacturing processes is required across the pharmaceutical industry to ensure the consistent production of a product to the required level of quality.

EXPERIMENTAL APPROACH:
The mapping of extraneous agents in raw materials, to high resolution, is an essential step in the Quality by Design process, and requires appropriate testing design and the inclusion of molecular techniques capable of detection of known and novel contaminants. Results from the evaluation of novel testing technologies will be presented, highlighting the most appropriate technologies for evaluation of raw materials, and the drawbacks of comparable technologies, specifically for the identification of extraneous viruses.

RESULTS AND DISCUSSION:
Case studies regarding the discovery of several viruses in animal sera and cell lines will be presented, and will be considered in the context of current regulatory recommendation and guidelines for testing. Strategies for routine testing to mitigate risk of extraneous agents in raw materials will also be presented.

A NEW PLATFORM OF MILLI-BIOREACTORS FOR ANIMAL CELL CULTURE

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KEY WORDS:
MILLI-BIOREACTORS / SCALE-DOWN / CELL CULTURE

BACKGROUND AND NOVELTY:
Today, there is an increasing demand for small-scale bioreactors for animal cell culture as useful tool for scale-down systems. By reducing culture medium volumes, more experiments can be managed in parallel, culture costs are reduced, especially for screening or hydrodynamics effects study purpose. Moreover, these systems are especially needed when cell quantities are limiting, for example in the case of stem cells. However, most of the small scale bioreactors such as shaken micro-well plates or shaking flasks do not exhibit a geometric homothety with pilot or production scales. Thus, the hydrodynamic environment may sensibly vary from the lab scale to production scale entailing production variability. To fill this gap, we have designed and built a platform of six 250 mL milli-mechanically stirred bioreactors for animal cell culture with a minimal liquid volume of 50 mL.

EXPERIMENTAL APPROACH:
The milli-bioreactors can be equipped simultaneously with three standard sterilizable probes such as oxygen, pH, CO2 or biomass probes. Marine propellers, Rushton turbines and ear-elephant impellers are available to ensure liquid mixing and mass transfer. A porous sintered cylinder is used to sparge air or pure oxygen bubbles in the liquid. A dedicated software allows the control of the 6 bioreactors independently.

RESULTS AND DISCUSSION:
The design of the milli-bioreactors was based on the scale-down of a standard 2-L bioreactor on the basis of various criteria. First a geometric homothety was imposed so that agitation and aeration criteria could be relevant. The volumetric oxygen transfer coefficient kLa was maintained by keeping constant the power dissipation per unit of volume and the superficial gas velocity. Consequently, averaged and maximal dissipation rate and thus hydrodynamic stresses range are supposed to be similar between the two lab-scale bioreactors. The first kinetics studies performed in the milli-bioreactors have confirmed their ability as scale-down bioreactor for animal cell culture.

APPROACHES TO DEVELOP AND CHARACTERIZE AN IMPROVED COMMERCIAL CHO CELL CULTURE PROCESS WITH NOVEL PROCESSING CONDITIONS TO ACHIEVE COMPARABILITY

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KEY WORDS:
CHO / GLYCOSYLATION / SIALIC ACID / PROCESS CHARACTERIZATION

BACKGROUND AND NOVELTY:
Understanding cell culture parameters that affect protein glycosylation has been a challenging area of investigation for decades. Results continue to be relevant because cell culture engineers must ensure product comparability and demonstrate manufacturing consistency. These goals are especially important when improving commercial processes with previously established specifications for product quality attributes.

EXPERIMENTAL APPROACH:
An improved CHO cell culture process was developed from an existing commercial process. Titer was improved while removing animal-derived raw materials and providing better facility fit for future process transfer. Although most product quality attributes were comparable, a higher than typical sialic acid content was observed. Experiments were conducted to understand which parameters were contributing to the high sialic acid content. Specific conditions were chosen based on knowledge of biosynthetic and degradative glycosylation pathways. After conditions were finalized, the process was transferred to various sites and scales. Small-scale experiments were also conducted to characterize parameter ranges.

RESULTS AND DISCUSSION:
Media components and process parameters were identified that consistently affected sialic acid content while monitoring other product quality attributes, such as deamidation and mannose-phoshate content. Comparable sialic acid content was achieved using low pH conditions that releases and activates sialidase, an endogenous CHO enzyme. At small-scale, multivariate and univariate experiments were combined to design an overall process characterization strategy. Process parameters were chosen using a risk based approach, and test ranges were proposed by stochastic simulations utilizing effect estimates from process development data. Ranges were meant to be sufficiently broad without risking excessive number of results outside established specifications. The new, characterized process has been successfully demonstrated at multiple sites and scales.

REF-A049

NEXT GENERATION HUMAN ALPHA INTERFERONS OBTAINED IN CHO CELLS BY O-GLYCOENGINEERING

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KEY WORDS:
O-GLYCOENGINEERING / INTERFERON / ANIMAL CELLS / GLYCOSYLATION

BACKGROUND AND NOVELTY:
Multiple biological activities of human alpha interferons (hIFN-) including antiviral, antiproliferative and immunomodulating properties have motivated the development of their recombinant forms to be used for treatment of numerous viral and tumor diseases. Unfortunately, one major issue regarding the clinical use of rhIFN- is its short half-life in circulation after injection of patients. For that reason, administration of high and frequent doses of the cytokine is required, leading to many adverse side effects.

Our goal was to exploit the ability of O-glycans to extend the half-lives of proteins to create O-glycosylated rhIFN- variants with lower in vivo clearance and preserved bioactivity.

EXPERIMENTAL APPROACH:
For this purpose, we fused a peptide containing four potential O-glycosylation sites derived from the carboxi-terminal sequence of the hCG -subunit (CTP) to the N-terminal and the C-terminal ends of rhIFN- 2b. CHO-K1 cells were employed as hosts to express CTP-IFN and IFN-CTP for physicochemical and biological characterization.

RESULTS AND DISCUSSION:
For both molecules SDS-PAGE followed by Western blot evidenced the presence of a broad band between 28 and 37 kDa, indicating a great microheterogeneity of glycoforms with higher molecular mass with respect to wild-type IFN. This result was confirmed by isoelectric focusing/Western blot, which clearly showed a great number of isoforms with highly acidic pI due to the incorporation of the CTP and, consequently, of new carbohydrates containing terminal sialic acids. In vitro antiviral specific bioactivity of CTP-IFN and IFN-CTP ranged between 66 and 74%, respectively, compared with that of native rhIFN- 2b, while antiproliferative specific bioactivity remained practically invariable (92 and 112%, respectively).

These preliminary results suggest that O-glycoengineering could be an attractive approach in order to increase charge and mass of IFN with the aim of improving its pharmacokinetic properties while preserving high in vitro bioactivity.

REF-A050

FEEDING STRATEGY OPTIMIZATION IN INTERACTION WITH TARGET SEEDING DENSITY OF A FED-BATCH PROCESS FOR MONOCLONAL ANTIBODY PRODUCTION

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KEY WORDS:
FED-BATCH / QBD / MONOCLONAL ANTIBODY / FEEDING STRATEGY OPTIMIZATION

BACKGROUND AND NOVELTY:
Current trend towards Quality by Design (QbD) leads the process development exercise towards systematic experimentation, rational development, process understanding, characterization and control. In this poster, an example of the application of QbD approach is given to enhance monoclonal antibody (mAb) titer and to ensure quality consistency of the product.

EXPERIMENTAL APPROACH:
A fed-batch process in 2L scale was run with different daily fixed volume feed additions. In this experiment, a correlation between feeding strategy and specific mAb productivity was observed. A custom Design of Experiment (DoE) with a statistical software was then performed on feeding strategy in growth phase, feeding strategy in production phase and day of change of feeding strategy. DoE allowed to reduce the number of required experiments to a reasonable number while maintaining statistically significant results. A second custom DoE was performed to further optimize the feed strategy and study the impact of an additional parameter the target seeding density (TSD).

RESULTS AND DISCUSSION:
In the process runs performed with different daily fixed volume feed additions, it was observed that a significant decrease in the specific mAb productivity occurred if the feed ratio per the projection of a subset of process performance attributes was below a specific threshold. A feed addition strategy based on this projected subset of process performance attributes was therefore developed. The feed ratio and the feed volume before day of changing strategy were two parameters significantly impacting mAb titer at harvest. It was shown that the higher they were, the higher was the titer. Thus it was decided to investigate the impact of greater feed ratio in interaction with the TSD. Feed ratio and the interaction feed ratio*TSD had a significant impact on mAb titer at harvest. The higher they were, the higher was the titer. ANOVA analysis showed that a 36% increase in the mAb titer was obtained in the optimized conditions compared to control condition.

PROCESS DEVELOPMENT AND OPTIMIZATION OF FED-BATCH PRODUCTION PROCESSES FOR THERAPEUTIC PROTEINS BY CHO CELLS

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KEY WORDS:
PROCESS DEVELOPMENT

BACKGROUND AND NOVELTY:
In the biopharmaceutical industry, process development and optimization is key to produce high quality recombinant proteins at high yields. As technologies mature, pressure on cost and timelines is greater for delivering scalable and robust processes.
Overall, process development should be viewed as a continuum from the early stages up to process validation. Here we outline a lean approach on upstream development during the initial phases to optimize yields while maintaining the desired product quality profiles.

EXPERIMENTAL APPROACH:
Early-stage process development was designed to lead to the establishment of a baseline process and to systematically include experiments with input parameters that have a high impact on performance and quality. At this stage, potential for pre-harvest titer and yield increases as well as product quality challenges were identified. Feed adjustments and systematic experiments with top, high and medium impact parameters have then been performed to develop a robust and scalable process. This approach was applied to two early stage upstream processes.

RESULTS AND DISCUSSION:
A baseline process was established and optimization of the feeding strategy was then performed. The feed regime was further optimized in combinatorial studies with an additional parameter: the target seeding density. In parallel, the impact of the process duration was also assessed.Then, the design of the feeding strategy was simplified in order to facilitate the process transfer to larger scale facility. Feeding, pH and temperature ranging studies and mode of feed addition were also performed in small scale due to facility fit considerations in the context of scale up. Consolidation runs were carried out and the robustness of the processes was assessed by performing over- and underfeeding experiments. For both projects, high titers and quality consistency were achieved with a feeding strategy designed to be robust and scalable.

PRODUCT QUALITY LESSONS LEARNED FROM DEVELOPING AND IMPLEMENTING A CHEMICALLY-DEFINED CHO PLATFORM CELL CULTURE PROCESS

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KEY WORDS:
CELL CULTURE PLATFORM / CHEMICALLY DEFINED MEDIA / PRODUCT QUALITY

BACKGROUND AND NOVELTY:
Many biopharmaceutical companies have developed cell culture platform processes for the production of recombinant monoclonal antibodies in mammalian cells. The use of platform processes for the production of clinical material has several advantages including lower costs for process development and faster generation of clinical material, thus enabling a reduced timeline to entry into clinical studies. Considerable efforts in the industry have been directed towards the development of chemically defined media for industrial cell culture processes. The use of such media reduces or eliminates some of the risks associated with the use of hydrolysate containing media, including inconsistent performance due to lot to lot variability, potential to introduce adventitious agents, raw material sourcing. During the development and implementation of our first chemically defined CHO cell culture process platform we observed some changes in mAb product quality profiles compared to results obtained in our older platform process using hydrolysate containing media.

EXPERIMENTAL APPROACH:
Cell culture experiments aimed at identifying media components that could be responsible for the observed changes in product quality (charge variants, drug substance color) were conducted using several cell lines expressing different mAbs. Different concentrations of certain media components or component groups were evaluated and product quality was assessed.

RESULTS AND DISCUSSION:
Our studies led to the identification of several media components that can have a significant effect on several product quality attributes. Copper and zinc were identified as key components that can affect the presence of basic variants (proline-amidation, C-terminal lysine). Certain vitamins and iron were identified as levers that can be used to manipulate drug substance color. This presentation highlights that the use of chemically defined media enables us to better understand our processes (and observed challenges) and will ultimately lead to better process control.

REF-A053

SCALE-UP CONSIDERATIONS FOR MONOCLONAL ANTIBODY PRODUCTION PROCESSES

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KEY WORDS:
SCALE-UP / STIRRED TANK BIOREACTOR / OXYGEN TRANSFER FLUX

BACKGROUND AND NOVELTY:
When scaling up a monoclonal antibody production process in stirred tank bioreactor, oxygen transfer is probably one of the most challenging parameters to consider. Approaches such as keeping constant specific power input or tip speed across the scales are widely described in the literature and are often based on the assumption that mammalian cells are sensitive to shear stress.
However, with the high cell densities reached in modern processes, such scale-up strategies can lead to relatively high gas flow rate to compensate low agitation speed which could be detrimental to cells in its own right.
As an alternative, we explored a scale-up strategy based on the overall oxygen transfer flux (OTF) required by the cell culture process. OTF was defined as directly proportional to oxygen transfer coefficient (kLA) and oxygen enrichment in the gas mix. This way the overall gas flow can be kept at low values, while satisfying the oxygen requirements of a high cell density culture.

EXPERIMENTAL APPROACH:
The maximum OTF associated with a model process was calculated according to the following equation:
OTF = kLA x (% of oxygen in gas mix) / 20
Aim was to scale-up a fed-batch process developed at in 10L glass bioreactors to a 80L stainless steel bioreactor.
kLA measurements were performed on the 2 systems and were considered together with the capability of the equipments to enrich gas with oxygen.

RESULTS AND DISCUSSION:
From the kLA mapping, different combinations of agitation speed / gas flow rates / oxygen enrichment were determined in order to achieve the desired OTF. Cell culture results showed that a wide range of agitation speeds was well tolerated by the cells, However, high gas flow rates negatively impacted cell culture performance.
Using constant maximum OTF as a main criteria, scale-up from 10L to 80L bioreactor of our process was performed. Relatively high agitation speed was applied while maintaining gas flow rates low, but sufficient to ensure CO2 removal, and using a gas mix highly enriched in oxygen.

REF-A054

DIFFERENCES IN THE PRODUCTION OF HYPERGLYCOSYLATED IFN ALPHA IN CHO AND HEK 293 CELLS

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KEY WORDS:
CHO CELLS / HEK CELLS / INTERFERON / N-GLYCOSYLATION / PROTEIN EXPRESSION

BACKGROUND AND NOVELTY:
IFN4N is an IFN-alpha2b mutein developed in our laboratory using glycoengineering. This molecule contains 4 potential N-glycosylation sites, resulting in higher apparent molecular mass and longer plasmatic half-life compared to non glycosylated IFN-alpha used for clinical applications. CHO cells are widespread used for the large-scale production of therapeutic recombinant proteins and HEK 293 cells are an interesting system for the generation of recombinant cell lines because they are easy to transfect and, consequently, they allow the production of high levels of the protein of interest.

EXPERIMENTAL APPROACH:
In this work, lentiviral vectors containing the sequence of IFN4N were assembled and used for transduction of CHO and HEK 293 cells. The recombinant cell lines were subjected to a process of selective pressure using increasing concentrations of puromycin. After cloning, 6 clones were selected for the study of their growth, production parameters and characterization of the secreted IFN4N.

RESULTS AND DISCUSSION:
The CHO and HEK IFN4N producing cell lines resistant to the highest concentration of puromycin showed an 8-fold and 15-fold increment in IFN4N specific productivity, respectively, compared to the parental line. HEK clones exhibited lower μ and maximum cell density than CHO clones, but higher IFN4N cumulative production was achieved. Significant differences in the glycosylation pattern of IFN4N produced in CHO (CHO-IFN4N) and HEK293 (HEK-IFN4N) were observed by isoelectric focusing followed by western blot. Specifically, IFN4N produced in CHO cells showed more acidic isoforms than the one produced in HEK. Furthermore, this result was consistent with a lower in vitro antiviral and antiproliferative specific biological activity evidenced by the CHO-IFN4N compared to the HEK-IFN4N.

Considering that glycosylation affects protein stability, solubility, pharmacokinetics and immunogenicity, differences between CHO and HEK cells should be capitalized to select the proper system for the cytokine´s production.

KEY ASPECTS FOR A SMOOTH TRANSITION FROM DEVELOPMENT TO COMMERCIALIZATION OF A MONOCLONAL ANTIBODY PRODUCTION PROCESS

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KEY WORDS:
MONOCLONAL ANTIBODY PRODUCTION / PROCESS CHARACTERIZATION / CRITICALITY ASSESSMENT

BACKGROUND AND NOVELTY:
For the production of therapeutic monoclonal antibodies it is mandatory to demonstrate proper control of a manufacturing process, thus ensuring that the biological product consistently meets its desired quality attributes and specifications. For process developers this means rational and systematic process development and process definition with thorough understanding of the impact of process parameters on product quality attributes. However in a commercial environment the Time to Market is also key, therefore lean but high quality process development is required and should be viewed as a continuum from early stage development to process validation. Process characterization is the final stage linking development and commercial manufacturing. Here we outline a roadmap for an upstream process and give examples for specific stages of the pre-validation phase.

EXPERIMENTAL APPROACH:
In parallel with scale down model establishment, a failure mode and effects analysis (FMEA) was performed in collaboration with subject matter experts from development, technology transfer, quality assurance and manufacturing. This powerful risk prioritization tool involved three orthogonal risk evaluations: severity, occurrence and detectability. This enabled the identification of potentially key and/or critical parameters and prioritization of follow-up activities.

RESULTS AND DISCUSSION:
Based on the outlined approach we identified the parameters to be studied at laboratory scale. Once the experimental work was completed, process performance and product quality data were used to achieve four goals:

1. Enhance in-house understanding of the process
2. Re-assess severity of the individual parameter failure mode
3. Establish PARs (Proven Acceptable Range) and NORs (Normal Operating Range)
4. Define criticality of the given parameter using an in-house criticality tree

We reduced the NOR of parameters that showed a direct and significant impact on product quality within the tested ranges to reduce the risk of falling outside of the PAR.

ADDRESSING PRODUCT QUALITY CHANGES DURING LATE STAGE CELL CULTURE PROCESS DEVELOPMENT

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KEY WORDS:
CHO / THERAPEUTIC PROTEIN / SEQUENCE VARIANTS / PROCESS CHARACTERIZATION

BACKGROUND AND NOVELTY:
The cell line and cell culture process used for late stage projects may differ from those used early in clinical development. Decisions to change the cell line, process or both are based on several considerations. One common reason for making such change between early and late stage projects is that a more productive cell line may be warranted based on forecasted commercial demand. Another reason for change between early and late stage is that the production platform used for initial development has evolved or has been optimized from the previous state. These changes may lead to variation in product quality attributes from early development experience.

EXPERIMENTAL APPROACH:
Experiments were carried out using Genentech's current production platform and bioreactor scale down model.

RESULTS AND DISCUSSION:
The case study presented here describes the challenges associated with observed product quality differences between the new process which implemented a higher productivity cell line and chemically-defined media. Studies were completed to minimize the product quality changes initially observed including the prevention of amino acid substitutions (sequence variants) in the antibody product.

REF-A057

IMPLEMENTING PFIZER'S BIONET DELTA V BIOREACTOR CONTROL SYSTEM

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KEY WORDS:
PROCESS DEVELOPMENT / BIOTHERAPEUTICS / BIOREACTOR / EFFICIENCY / PROCESS UNDERSTANDING

BACKGROUND AND NOVELTY:
In this oral presentation, we describe the systematic approach taken by Pfizer's BioTherapeutics Pharmaceutical Sciences Bioprocess R&D to identify, evaluate, and implement the company's next generation bioreactor control system. A five year commitment in resource investment (capital and FTE) and strategic partnership with key bioprocess technology vendors was necessary to achieve this goal.

EXPERIMENTAL APPROACH:
The endeavor will be described in a time-line of events and three distinct phases will be highlighted: Technology Evaluation (Phase 1), Technology Maturation (Phase 2), and Technology Deployment (Phase 3). Key team challenges/learnings and solutions to work through for each phase will be shared.

RESULTS AND DISCUSSION:
The result of this effort is a bioreactor control system that drives efficiency in the PD laboratories, increases data capture capability reinforcing process development towards QbD (Quality by Design), and provides a flexible structure to integrate new emerging bioprocess technologies to support process understanding.

REF-A059

TECHNOLOGY TRANSFER AND SCALE DOWN MODEL DEVELOPMENT STRATEGY FOR BIOTHERAPEUTICS PRODUCED IN MAMMALIAN CELLS

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KEY WORDS:
CELL CULTURE / BIOPROCESS / SCALE DOWN MODEL / TECHNOLOGY TRANSFER

BACKGROUND AND NOVELTY:
The goal of a manufacturing process development for drug substance is to establish a commercial manufacturing process capable of consistency producing drug substance of the intended quality. Based on the regulatory requirements, the manufacturing process has to be characterized prior to process validation. Since performing the characterization study at the manufacturing scale is not practically feasible, development of a scale down model that represents the performance of the commercial process is essential to achieve reliable process characterization. This study describes the methodology applied to ensure a successful scale down development and the associated technology transfer required prior to the establishment of the small scale process.

EXPERIMENTAL APPROACH:
The scale down model is developed by identifying volume-dependent parameters and volume-independent parameters. A systematic method to prioritize operating parameters and to evaluate their potential impact to the process is the Failure Mode and Effect Analysis (FMEA). An enhanced approach to manufacturing process development will then include a process characterization study (PCS), a systematic investigation to understand the commercial scale process in detail including relationship between key operating parameters and the cell culture performance for growth, productivity and the product quality attributes.

RESULTS AND DISCUSSION:
The information and knowledge gained from process development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, product specifications and manufacturing control. To ensure a successful scale down model establishment, the technology transfer has to elucidate necessary information and detailed documentation to be transferred by focusing on the handling variations of raw materials, raw material lead time and storage, sampling and testing, equipment scale differences, process flow diagram, process controls, data to be recorded.

TOWARDS UNDERSTANDING THE COMPLEXITY OF HYDROLYSATES

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KEY WORDS:
HYDROLYSATES / QBD / STATISTICS

BACKGROUND AND NOVELTY:
Hydrolysates are complex media supplements composed of many as well as different types of compounds. FrieslandCampina Domo's Quality by Design project has generated detailed information of these compounds (annotation and quantification) and cell culture performance data of many lots of Proyield soy SE50MAF-UF.
Using different statistical approaches, key compounds present in hydrolysates are identified that significantly correlate with cell culture performance. These compounds are demonstrated to interact with several other compounds in a complex biochemical network.
This network of compounds is a unique and native feature of hydrolysates and non-existent in chemically defined media. Addition of these individual key compounds to media in some cases slightly improves titer, but the effect is still much smaller than the effect of the complete hydrolysate. This suggests that the effect of a hydrolysate cannot be mimicked by adding key metabolites.
Working in close collaboration with our customers, we gain understanding about the relation between the complex composition of hydrolysates and their effect on cell growth and titer in the application.

EXPERIMENTAL APPROACH:
The Biochemical composition of soy hydrolysates (Proyield Soy SE50MAF-UF, Frieslandcampina Domo) has been identified by Metabolomics Biochemical profiling.
Biochemical profiling, together with peptide profiling and analysis of inorganic compounds, provides a complete characterization of this hydrolysate product.
By applying statistical tools which include Two-mode cluster analysis, Bootstrapped stepwise regression and 2D correlation analysis, a series of compounds in the hydrolysate were identified that correlate with cell growth or Production of IgG in a CHO cell line (key compounds).

RESULTS AND DISCUSSION:
The enhancing effect of some of these key components on specific production could be proven, but is still much lower than the enhancing effect of a hydrolysates supplement. 2D correlation analysis reveals a complex network of positive and negative.

DESIGN OF A MINI-BIOREACTORS PLATFORM BASED ON A SCALE-DOWN CHEMICAL ENGINEERING APPROACH

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KEY WORDS:
SCALE-DOWN / MINI-BIOREACTORS / HYDRODYNAMICS

BACKGROUND AND NOVELTY:
There is an increasing demand for small-scale bioreactors as tools for scale-down processes. By reducing culture volumes, more experiments can be managed in parallel and costs are decreased, especially for media screening or hydrodynamics effects study. Moreover, small systems are especially pertinent when cell availability is limited, as in the case of stem cells. However, most of the small-scale bioreactors (shaken well plates or shaking flasks) do not exhibit a geometric homothety with production scale. Thus, hydrodynamics may significantly vary entailing productivity variability. To fill this gap, our objective was to design and built a platform of six 250 mL mini-mechanically stirred bioreactors by using a chemical engineering scale-down approach.

EXPERIMENTAL APPROACH:
Specifications required that mini-bioreactors could be equipped simultaneously with three sterilizable probes (dissolved O2, pH, CO2 or biomass) and were usable with a minimal liquid volume of 70 mL. Marine propellers, Rushton turbines and ear-elephant impellers were provided to ensure various liquid mixing and mass transfers. Aeration could be done by a porous sintered cylinder, an open pipe or a perforated ring. A software allowed the control of the 6 bioreactors independently.

RESULTS AND DISCUSSION:
The bioreactors design was based on the scale-down of a standard 2-L bioreactor using various criteria. First, a geometric homothety was imposed so that agitation and aeration criteria could be relevant. Furthermore, the same volumetric oxygen transfer coefficient kLa was maintained by keeping constant the power dissipation per unit of volume and the superficial gas velocity. This was done by a theoretical approach based on a flow similarity hypothesis. Consequently, averaged and maximal turbulent dissipation rate and thus hydrodynamic stresses range are expected to be similar between the two bioreactors. The first kinetics studies performed in the mini-bioreactors have confirmed their ability as scale-down bioreactors for animal cell culture.

REF-A062

AN INTEGRATED SYNCHRONIZATION APPROACH FOR STUDYING CELL-CYCLE DEPENDENT PROCESSES OF MAMMALIAN CELLS UNDER PHYSIOLOGICAL CONDITIONS

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KEY WORDS:
SYNCHRONIZATION / MAMMALIAN CELL CULTURE / ELUTRIATION / DIALYSIS CULTURE

BACKGROUND AND NOVELTY:
The study of central metabolism and the interactions of its dynamics with growth, product formation and cell division is a key issue for decoding the complex metabolic network of eukaryotic cells. For this purpose, not only the quantitative determination of key cellular molecules is necessary, but also the variation of their expression rates in time, e.g. during cell cycle. The enrichment of cells within a specific cell cycle phase, or cell synchronization, should in this way allow for the generation of a cell population with characteristics required for cell cycle related studies.

EXPERIMENTAL APPROACH:
Using a combined approach, centrifugal elutriation was employed for synchronization in different cell cycle phases of two production cell lines. Cells were afterwards cultivated in benchtop bioreactors with culture volumes ranging between 200 mL and 1 L. A dialysis bioreactor (Bioengineering AG, Switzerland) with a total volume of 3.8 L was used for the cultivation of one cell line in order to allow for a higher number of synchronous cell divisions.

RESULTS AND DISCUSSION:
Our first results demonstrated the successful separation of a heterogeneous AGE1.HN® cell population into synchronous subpopulations [1]. These subpopulations showed a high degree of synchrony independently of the targeted cell cycle phase. Bioreactor culture showed no noticeable perturbation in the doubling time of the population. With these result, one of the most important requirements for Omics research was fulfilled. The dynamic behaviour of the synchronous growing cells was systematically studied not only based on cell growth, but also on the distribution of the cell size and the DNA content of the cells. Furthermore, dialysis culture allowed for a higher number of synchronous cell divisions without noticeable perturbations. With this contribution, we present an integrated approach for cell synchronization and further unperturbed cultivation which is useful for studying cell-cycle dependent processes under physiological conditions.

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REF-A063

MODEL-BASED DESIGN OF THE FIRST STEPS OF A SEED TRAIN FOR CELL CULTURE PROCESSES

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KEY WORDS:
PROCESS DEVELOPMENT / SEED TRAIN / MODEL-BASED DESIGN

BACKGROUND AND NOVELTY:
The production of biopharmaceuticals for therapeutic and diagnostic applications with suspension cells in bioreactors requires a seed train up to production scale. For the final process steps in pilot and production scale, the scale-up steps are usually de-fined (e.g. a factor of 5 - 10), so that the respective cell concentrations required for seeding and harvesting can be considered as similar. More difficult in this respect are the first steps, the transitions to T-flasks, spinner tubes, roller bottles, shake flasks, stirred bioreactors or single-use reactors, because here often the same scale-up steps cannot be realized. The experimental effort to lay-out these steps is corre-spondingly high. At the same time it is known that the first cultivation steps have a significant impact on the success or failure on production scale.

EXPERIMENTAL APPROACH:
In the present work, for a suspendable cell line (AGE1.HN, ProBioGen AG) basic kinetic information for cell growth and death, substrate uptake and metabolite production were generated in four well directed shake flask batches using different initial substrate and metabolite concentrations. Based on data of two batches, a Nelder-Mead-algorithm has been applied to determine the model parameters of an unstructured kinetic model. Using a MATLAB / Simulink simulation based on this model, optimal points of time or viable cell concentrations respectively for harvest of a seed train from spinner tubes over shake flasks up to a stirred bioreactor (5 L) were determined and subsequently verified experimentally. Model prediction for optimization and experiment agreed very well. To be able to judge the optimization, the optimization results have been compared to a formerly manually optimized seed train of the same cell line. Without such time consuming lab work, the tool has delivered the same optimized seed train only based on data of two batches.

RESULTS AND DISCUSSION:
The concept offers a simple and inexpensive strategy for design of the first scale-up steps.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Acknowledgement: The bioreactor (Labfors 5 Cell) was kindly provided by the company Infors AG, the cell line AGE1.HN by ProBioGen AG.

SHAKE FLASK CONDITIONS AND OPTIMISATION FOR CONSISTENT CHO CELL CULTURE GROWTH

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KEY WORDS:
SHAKE FLASK / SCALE-UP / DOE / CHO

BACKGROUND AND NOVELTY:
Shake flasks (SFs) are the most commonly used culture system on a millilitre scale. Shake flask are used during the development of a mammalian cell lines for a range of tasks including expansion of seed cultures and screening and evaluation of potential production cell lines using platform methods. In order to develop a robust cell line development programme or seed expansion process, the SF culture parameters that impact on cell line performance should be evaluated. Whilst optimisation of SF culture such that culture performance is representative of performance in bioreactors conditions is a focus of much research , other experimental aims are also important. For example, establishing conditions that result in robust, consistent and predictable growth across the working volume of a range of different shake flask sizes and configurations would improve scale up and technical transfer into cGMP manufacturing.

EXPERIMENTAL APPROACH:
Statistical-based factorial design methods were used optimise culture conditions which would provide robust and consistent growth of CHO cultures across the working volume of a range of different SF sizes. The impact of flask venting, agitation speed, SF working volume and total volume and flask design were evaluated. Culture growth, Monoclonal antibody and metabolite productivity and substrate utilisation were used as responses. These responses were optimised to maximise growth and monoclonal antibody productivity while maintaining the performance robustness and consistency across SF volumes.

RESULTS AND DISCUSSION:
Total and working volume were shown to have a large impact on growth and productivity. Differences in maximum IVC (Time Integral of Viable Cell Concentration) were observed initially in the range of ~ 280 to 380 x 106 cells/mL.h across the conditions evaluated. Conditions were identified which improved growth robustness and consistency across SF volumes. The use of vented flasks was shown to have a positive impact on growth consistency across SF volumes. The impact and exploitation of these improvements will be discussed.

CHARACTERIZING HETEROGENEITIES OF ENVIRONMENTAL CONDITIONS IN VARIOUS BIOREACTOR SCALES USED FOR MAMMALIAN CELL CULTURES

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KEY WORDS:
BIOREACTOR CHARACTERIZATION / BIOREACTOR UP- AND DOWN-SCALING / MAMMALIAN CELL CULTURES

BACKGROUND AND NOVELTY:
Large scale mammalian cell cultures up to 20000L have been established as an industrial standard over the last few years. Process up scaling to those volumes is a fundamental necessity to guarantee process performance and in particular product quality. Therefore, it is of up-most importance to be aware of scale dependent variables, i.e. reactor geometry, shear stress distribution, dissolved O2 and CO2 distribution, gas mass transfer rates and pH perturbations due to feeding policy and position of feeding ports, which can be very different in between scales and might result to non-desired process variations.

EXPERIMENTAL APPROACH:
Computational Fluid Dynamic (CFD) simulations were used to study scale dependent parameters (e.g. shear stress, kLa, DO, pCO2, pH perturbations and mixing time) covering a volume range from 3L up to 15000L., Good agreement of CFD simulations with experimentally measured data obtained at each scale supports applied approach. Consequently, the trajectory analysis was used to characterize heterogeneities of environmental conditions among various vessel scales.

RESULTS AND DISCUSSION:
The results were implemented into a rational scale-down model to mimic conditions present in large scale bioreactors. Excellent agreement between scale-down cultivation data and those obtained from large scale bioreactors support the proposed methodology. An indirect implication of this work was to identify physical limits of various environmental parameters to which cells respond in terms of growth, product quantity and quality. This new approach allows generation of a rational engineering design space as well as can be used for further process optimization and control of the process robustness.

REF-A066

IMPLEMENTATION OF A MICRO BIOREACTOR SYSTEM IN A PLATFORM FOR CELL LINE AND PROCESS DEVELOPMENT

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KEY WORDS:
MICRO BIOREACTOR / AUTOMATION / FED BATCH / PROCESS DEVELOPMENT

BACKGROUND AND NOVELTY:
Requirements for rapid cell line and process development have pushed more and more process runs to a higher throughput in smaller scale. Tight time lines are very important when developing a platform approach for an antibody program from cell line development to production of clinical material. Evaluation of cell culture media and additives can be very time consuming as well as process parameter optimisation. The aim of the implementation of a micro bioreactor system was therefore to find an approach where cell culture media and additives can be tested and at the same time have control over bioreactor process parameters to facilitate the scale up of the bioreactor process.

EXPERIMENTAL APPROACH:
The micro bioreactor system (Ambr) from TAP Biosystems was selected for rapid process development and cell line development. Cell culture performance will be compared to glass bioreactors as well as 250L SUB. Titer, protein quality and timeline for cell line selection will be evaluated.

RESULTS AND DISCUSSION:
Data will be shown
(1) where the feed strategy for a CHO-S cell culture process was optimised and the antibody concentration could be increased from 0.4 g/L to 2.7 g/L after three runs of optimisation in the Ambr system in less than two months.
(2) where evaluation of media and additives were performed in order to improve product potency by altering the glycosylation pattern.
(3) where the micro bioreactor system was useful during cell line development in evaluation of clone stability and protein expression. Feed strategy and clone screen could be evaluated simultaneously and thereby base the clone selection on bioreactor data where parameters such as pH and dissolved oxygen (DO) can be controlled instead of using traditional shake flask experiments.
The process performance in the micro bioreactor system was comparable to 250L scale which is very useful for a platform approach where an early estimation of process performance in production of clinical material is advantageous.

REF-A067

NEW IMPROVED AUTOMATION OF THE AMBRTM MICROBIOREACTOR IMPLEMENTED IN A CLONE SELECTION WORKFLOW

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KEY WORDS:
SCALE-UP MODEL / CLONE SELECTION / BIOPROCESSING / PH REGULATION / CELL LINE DEVELOPMENT

BACKGROUND AND NOVELTY:
Within the biopharmaceutical industry, it is increasingly recognised that the shake flask has significant limitations as a model for predicting large scale bioreactor performance of clones, largely due to lack of pH and DO control in the shake flask. In addition, the shake flask is unsuitable for high throughput screening and process development programs due to the high level of manual labour required to take samples and maintain cultures on a day-to-day basis. While bioreactor models are better, some manual interventions are required such as sampling for offline pH measurement and recalibration.

EXPERIMENTAL APPROACH:
A recent development in microbioreactor technology, the ambr™ system, has enabled high throughput bioreactor studies at the 10-15mL culture scale, with multiple reports of positive culture performance (1, 2, 3). However, some aspects of the culture maintenance have not been fully automated, such as offline pH recalibration and external calculation of feed volumes. In the high throughput ambr system, these tasks can become manually intensive. Here, for the first time, we investigate the capability of novel improvements in the ambr system, including both a new integrated device for automated pH recalibration and new software capability allowing complex feedback calculations.

RESULTS AND DISCUSSION:
These new elements of the ambr technology will be implemented in a candidate monoclonal antibody clone screening program, and the associated benefits will be analysed and discussed. It is anticipated that the study will demonstrate improvements in frequency and consistency of pH recalibration, improvements in pH control, and reductions in hands on staff time required to maintain the parallel microbioreactor cultures. Automated calculation and addition of feed volumes will also increase efficiency of the laboratory workflow, further reducing staff time requirements and so enabling larger high throughput studies to be carried out with existing laboratory and staff resources.

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SABIN-IPV PROCESS OPTIMIZATION FOR AN AFFORDABLE IPV IN THE POST POLIO-ERADICATION ERA

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KEY WORDS:
VERO CELLS / SCALE-DOWN / POLIOVIRUS / INACTIVATED POLIO VACCINE / ANIMAL-COMPONENT-FREE

BACKGROUND AND NOVELTY:
A production process for inactivated polio vaccine (IPV) using attenuated Sabin poliovirus strains was developed based on the current large-scale Salk-IPV production technology [1]. This activity for the WHO plays an important role in their polio eradication strategy since the use of attenuated Sabin poliovirus strains, instead of wild-type Salk strains, provides additional safety during vaccine manufacturing. Development of a new Sabin-IPV opens opportunities to implement improvements in the process. In this way, a more affordable IPV for the post-eradication era is strived for.

EXPERIMENTAL APPROACH:
To achieve these objectives, a scale-down – scale-up strategy was followed using historical manufacturing data. Based on this, a 2.3-L scale-down model of the current 750-L bioreactors has been setup. This lab-scale process, both USP (cell and virus culture) and DSP (clarification, concentration, purification and inactivation) unit-operations approximate the large-scale [2]. Subsequently, using this scale-down model, a modified process using attenuated Sabin poliovirus strains, was developed. This process was used to produce Sabin-IPV batches under cGMP for the currently ongoing phase I/IIa safety and dose-finding clinical trial in naïve infants [1].

RESULTS AND DISCUSSION:
In parallel, using the scale-down model, the process is being optimized to further reduce the cost per dose. The effect of three different Vero cell culture strategies, using animal-component-free cell- and virus culture media, on subsequent poliovirus production was investigated. Increased cell densities allowed up to 3 times higher D-antigen levels when compared with that obtained from batch-wise Vero cell culture. The increased product yields showed opportunities to reduce vaccine cost per dose by efficient use of bioreactor capacity. Further, the use of animal-component-free cell- and virus culture media showed opportunities for modernization of human viral vaccine manufacturing.

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OTHER INFORMATION
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WHY COMPLICATE, WHEN SIMPLE THINGS CAN MAKE BIG DIFFERENCES TO YOUR PROCESS

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KEY WORDS:
FED BATCH / PROCESS OPTIMIZATION / PROCESS DILUTION / INOCULUM MAINTENANCE / CHO

BACKGROUND AND NOVELTY:
Process improvement strategies for most fed batch culture fall under 3 categories: a. Cell line development methodologies, b. basal medium and feeds formulations, and c. Online process analytical techniques. Further process optimization is done by manipulation of typical process parameters like DO, pH, temperature etc. However there are certain small parameters that are rarely discussed during process optimization. In this poster, we will discuss 3 such parameters: Inoculum conditions, Common salts concentration in process and the total process dilution.

EXPERIMENTAL APPROACH:
Fed batch process was run with the following conditions in Shake flask and Bioreactor:
Case 1: Inoculum maintained at different pH, lactate, and osmolality.
Case 2: Common salts concentration in the process.
Case 3: Different levels of dilutions by feed addition. For all cases, multiple cell lines were studied and analyzed for cell conc., viability, glucose, lactate, osmolality.

RESULTS AND DISCUSSION:
No significant difference was observed during the inoculum stage when cells were maintained at different levels of pH, lactate and osmolality. However, about two fold increase in the cell concentration was seen in the production process favoring cell growth and maximum culture longevity across multiple CHO cell lines.
Common salts are largely used as an osmolality correction agent in medium formulation. In our studies we observed that common salts' concentration plays other roles that can significantly affect process performance.
Feed addition causes varying levels of dilutions in the process depending on feeding strategy. It is expected that higher dilution decreases total cell concentration and titer. However, within a certain range, we observed an increase in total cell concentration, productivity and better product quality profile with increase in dilution.
Thus simple factors as illustrated by the above cases can impact process performance in a big way. Paying attention to these simple factors can help in developing an efficient process.

REF-A070

PLATFORM PROCESS WILL GIVE PLATFORM PRODUCT – CAN WE AFFORD IT?

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KEY WORDS:
PLATFORM FED BATCH PROCESS / CLONE SELECTION / PRODUCT QUALITY / MABS / CHO

BACKGROUND AND NOVELTY:
As cell culture technology is maturing, there is a drive towards developing platform processes. The typical components of a platform process consist of cell line development, basal medium and feed strategy, process parameters and scale up approach. Some prominent benefits are shorter development timelines, facility fit, use of historical data, inventory management and easier integration with downstream process.

In our experience, the platform approach works well when target mAbs can have any levels of product quality (PQ) attributes like galactosylation and charge variant but may not work for mAbs with specific target for PQ.

EXPERIMENTAL APPROACH:
Following 3 cases will be discussed: 1. Multiple Cell lines expressing different mAbs developed using same technology 2. Difference in lead clone selection criteria – growth versus specific productivity and 3. Cell lines expressing same mAb developed using different technology.

For these cases, fed batch runs were performed using platform process in the shake flasks and lab bioreactors. Samples were measured for growth, productivity, metabolites and PQ attributes.

RESULTS AND DISCUSSION:
Case1: All cell lines showed similar growth and PQ profiles. Case2: The cell lines selected using different criteria showed two distinct cell growth patterns. Significant higher cell growth was observed for clones selected using the growth-based selection criteria in comparison to productivity based selection. However, PQ from these cell lines was comparable. Case3: The cell lines as expected showed differences in cell growth and metabolite profiles. These differences significantly impacted PQ attributes.
In all cases, the platform process had to be modified to achieve growth and PQ targets.
Our studies suggest that to produce mAbs with specific growth and PQ targets, platform process approach may not be appropriate as significant process changes may be needed to achieve these goals. We developed multiple platform processes to overcome illustrated disadvantages.

REF-A071

APPLICATIONS OF BIOMASS PROBE AS A PAT TOOL

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KEY WORDS:
PROCESS ANALYTICAL TECHNIQUES (PAT) / BIOMASS PROBE / FED BATCH / PERFUSION / CHO

BACKGROUND AND NOVELTY:
Cell culture process requires continuous monitoring of cell conc. and viability. Common techniques used apart from manual cell counting are automated cell counter and biomass probes. Unlike automated cell counters, biomass probe offer continuous monitoring of cell growth in the process. However, a major disadvantage of this technology is its correlation with cell size and morphology as it changes during the process. In this poster we will present usefulness of biomass probe in spite of above disadvantages.

EXPERIMENTAL APPROACH:
Cell conc. was estimated by automated cell counter (Vi-cell and Cedex) and compared with the readings of a biomass probe (Aber) to establish a correlation. This was done for both fed-batch and perfusion process in seed and production stages. For the perfusion process, an additional probe was inserted in the perfusate line to monitor the cell retention of the perfusion device.

RESULTS AND DISCUSSION:
The correlation between biomass probe readings and actual cell conc. was observed to decrease with increased process duration and drop in cell viability. Two cases are presented where biomass probe has advantages over traditional offline sampling and can be used as an effective PAT tool.
Case 1:Process consistency- Transfer of cells during seed stages is done based on cell conc. to maintain the log growth phase. For cell lines having high growth rate, biomass probe was found to be a better technique for such transfers leading to process consistency. Similarly in the production run, process consistency was improved by using biomass probe to trigger process events like feeding.
Case 2:Improvement in perfusion process- In our process, loss in retention in the perfusion device led to decrease in cell conc. and productivity. By monitoring retention continuously, corrective actions could be taken to reduce these losses. Introducing a biomass probe in the perfusate line overcame operational constraints of sampling continuously to monitor retention efficiency which led to improved process performance.

CHEMICAL MODIFICATIONS OF RAW MATERIALS IN DRY POWDER MEDIA FORMULATIONS TO SIMPLIFY CHO FED-BATCH PROCESSES

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KEY WORDS:
CHEMICALLY DEFINED MEDIA / SOLUBILITY / FED-REGIME / TYROSINE / CYSTEIN

BACKGROUND AND NOVELTY:
The solubility of e.g. L-Tyrosine and L-Cystein in chemically defined cell culture media is a limiting factor at neutral pH. This requires the usage of an additional feed at basic pH with all bioprocess related limitations like local pH spots resulting in cell death followed by cytoplasmic protein release supporting CHO cell aggregation and cell clumping.

EXPERIMENTAL APPROACH:
This can be circumvented by using modified molecules allowing for solubility at neutral pH in one main feed at concentrations with more than 50 g per liter of e.g. L-Tyrosine. We demonstrate that these novel moieties are metabolized by CHO cells and show no effect on the NBE level as this highly conserved t-RNA coordinated translation process uses unprocessed L-Tyrosine only.

RESULTS AND DISCUSSION:
This novel amino acid allows in combination with L-Cystein for a simplification of the fed batch regime without the necessity to add extreme pH solutions to the bioreactor.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Dr. Jörg von Hagen is head of Merck Millipore Process Development R&D in Darmstadt (Germany). Having studied biotechnology and signal transduction in Giessen and Darmstadt, he received his academic degree with an award-winning thesis in 2001. Dr. von Hagen has more than 20 years of practical expertise in biotechnology, especially in molecular cell biology and proteomics.

BI-HEX®: INNOVATIONS IN BOEHRINGER INGELHEIM'S INTEGRATED CELL CULTURE PROCESS DEVELOPMENT

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KEY WORDS:
CELL CULTURE / PROCESS DEVELOPMENT / NEW MOLECULAR FORMAT / CELLULAR ENGINEERING INNOVATION

BACKGROUND AND NOVELTY:
BI-HEX® is Boehringer Ingelheim's integrated cell culture platform used for developing and manufacturing processes. The CHO-based BI-HEX platform combines in one concept state-of-the-art technologies within vector design, cell line generation, process and media optimization. Strategies are in place for introduction of a new generation of media, shortening of seed expansion times, improved process feeding, and introduction of process formats and cellular modifications to control product quality. Innovations to accomodate newer molecule formats, higher performance benchmarks in process time and resources, and more stringent product quality requirements will be highlighted.

EXPERIMENTAL APPROACH:
Multiple tools were used ranging from systems biotechnology involving metabolic flux analysis and controlled feeding to statistical experimental design and multivariate analysis. Vector and cell line engineering have been other focus of activities. Newer advancements include exploration of controlled varying feeding based on online monitoring of cell growth.

RESULTS AND DISCUSSION:
Standardized workpackages and lean project structures have been implemented that allow reduced development and production cycle times. Process modifications including a balanced chemically defined medium and an advanced feeding approach have led to significantly higher growth and titers. Product quality matching has been enhanced via development of a standardized work package involving high throughput exploration of the design space. Cellular and process levers implemented allow for control on product quality attributes like ADCC activity.

REF-A074

SCALE TRANSLATION OF A 24-WELL MINIATURE BIOREACTOR AND SUBSEQUENT IMPACT ON PRODUCT AND BROTH QUALITY

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KEY WORDS:
MINIATURE SHAKEN BIOREACTOR / CHO CELL CULTURE / ANTIBODY PRODUCTION / PRODUCT QUALITY / ULTRA SCALE-DOWN (USD) PRIMARY RECOVERY

BACKGROUND AND NOVELTY:
To accelerate cell culture process development, most companies have validated scale-down models of their pilot and manufacturing scale bioreactors. Advancing such mimics to even smaller scales requires the large scale engineering environment to be accurately recreated. Here we describe a shaken microwell methodology that accurately reproduces not only cell growth kinetics but also key attributes related to product quality and broth processability.

EXPERIMENTAL APPROACH:
The micro24 bioreactor system enables system level control of agitation, with individual well control of pH, DO and temperature. Two distinct plate types are investigated, allowing for either headspace or direct gas sparging. An engineering characterisation was performed evaluating fluid mixing, gas transfer capacity and the dispersed gas phase. Cell culture is investigated using a model CHO cell line expressing a whole IgG1 mAb.

In addition, this work describes scale-up of micro24 results to laboratory scale stirred tank bioreactors (2L) and use of the device for selection of robust and scaleable cell lines through evaluation of product quality; and broth quality as evaluated by primary clarification efficiency using a USD depth filtration rig.

RESULTS AND DISCUSSION:
Apparent kLa values ranged between 3–22 hr^-1 and 4–53 hr^-1 for headspace aeration and direct gas sparging respectively. Mixing times (tm) were generally in the range 1–13 seconds and decreased with increasing shaking frequency (500–800 rpm). Direct gas sparging also helped to reduce tm values.

Cultures performed with headspace aeration showed the highest VCD and antibody titres, whereas those operated with direct gas sparging showed cell growth kinetics and product titres that were more comparable to those found in a conventional 2L stirred bioreactor. Initial results also indicate that key product and broth processability attributes are maintained making the combination of micro24 and USD technologies useful tools in 'Quality by Design' driven cell culture process development.

REF-A075

COMPARATIVE STUDY OF CHEMICALLY DEFINED CELL CULTURE SUPPLEMENTS FOR BIOPROCESS APPLICATIONS

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KEY WORDS:
SCALE-UP / CHEMICALLY DEFINED (CD) / FEED / SUPPLEMENT / TITER

BACKGROUND AND NOVELTY:
Throughout biopharmaceutical process development, many decisions are made in the production of biological therapies. Each biopharmaceutical process requires a unique cell culture environment for optimized performance. One bioprocess challenge is the selection and optimization of a cell culture supplement or feed that will give desired titer, growth characteristics, and protein quality. Therefore, the availability of a family of chemically defined (CD) supplements offering a diverse performance profile can significantly improve process development timelines. BD has developed a CD supplement pack to address this issue. The current studies were performed to determine the comparability of these supplements against other commercially available CD supplements or feeds, as well as their scalability in larger-scale processes.

EXPERIMENTAL APPROACH:
Three in-house mAb-producing CHO cell lines were used in these experiments. The comparative study was executed using five BD CD supplements and multiple commercially available CD supplements to evaluate their effect on production, growth, and viability. Shaking deep wells and conventional shaker flasks were used in these studies. The scalability of the five BD CD supplements was tested in small scale 1L bioreactors.

RESULTS AND DISCUSSION:
In testing on three CHO cell lines, protein yields of BD CD supplements were superior to results obtained with commercially available supplements. In addition, the BD supplements were seen to be suitable for use in small-scale bioreactors. The five BD supplements produced a 2-3-fold increase in protein production over media control in the 1L bioreactors. Equivalent results were obtained in shake flask studies during the development of these five BD CD supplements, hence demonstrating successful scalability. The growth characteristics were comparable or slightly better in 1L bioreactors compared to shake flasks.

DEVELOPMENT AND OPTIMIZATION OF A SET OF CHEMICALLY DEFINED MEDIA SUPPLEMENTS FOR MULTIPLE CHO CELL CULTURE SYSTEMS

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KEY WORDS:
CHEMICALLY DEFINED (CD) / FEED / SUPPLEMENT / PROCESS DEVELOPMENT / ANIMAL-FREE

BACKGROUND AND NOVELTY:
In biopharmaceutical production, optimization of cell culture parameters is a central component of process development. Every cell line can offer unique challenges for devising processes that yield desired growth profiles, high titers, and suitable product quality. Usually many different growth conditions must be tried before process goals are met. During the optimization process, the use of culture supplementation is often attempted for boosting performance, and this aspect of development can also benefit from having a diverse set of formulations to choose from.

EXPERIMENTAL APPROACH:
Three monoclonal antibody-expressing CHO cell lines were used for development and testing of animal-component-free, chemically defined (CD) formulation candidates, using CD basal media in all studies. Shaking deep well and conventional shaker flask formats were used during development. Formulations were generated using knowledge and analysis of cell line nutritional requirements, and design-of-experiments studies were carried out to optimize component levels. Formulation screening was accomplished using both batch and fed-batch modes.

RESULTS AND DISCUSSION:
The set of top formulations was selected during development for further commercialization. This set enhanced recombinant protein production in the test cell lines while retaining suitable product quality. As no single supplement was ideal for enhancing performance across all conditions, the need to test several supplements to cover a wider range of formulation space during process development was evident. In addition to being chemically defined, the supplement formulations are fully animal-free and protein-free, simplifying compliance with regulatory requirements. These results suggest that this collection of BD supplements can serve to enhance the quality and performance of bioprocesses across a diverse set of cell lines and growth conditions.

RELEVANCE OF YEAST EXTRACT FRACTIONATION BY CROSS-FLOW NANOFILTRATION TO SUPPLEMENT CHO CELL CULTURE MEDIUM

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KEY WORDS:
YEAST EXTRACT / NANOFILTRATION / CULTURE MEDIUM / CHO CELLS

BACKGROUND AND NOVELTY:
Yeast extract (YE), which is a soluble fractions of yeast autolysates, is known to greatly enhance mammalian cell culture performances, but their undefined composition decreases process reliability. Accordingly, it appears necessary to simplify YE composition and to better investigate the properties of molecules presenting a positive stimulating effect. This study aims to implement a process of YE fractionation by cross-flow nanofiltration, then to analyze the fractions composition and to evaluate their influence on CHO cell growth.

EXPERIMENTAL APPROACH:
The nanofiltration process was performed in a ProScale system (Millipore), using a spiral-wound Nanomax 50 membrane (500 Da, 0.4 m2). YE and fractions were characterized by their composition in amino acids, carbohydrates, nucleic acids and by their peptides molecular size distribution [1]. Trehalose was assayed by an enzymatic kit (Megazyme) and r-IgG by ELISA. CHO-AMW were cultivated inside 125 mL shake flasks in reference BDM serum-free medium supplemented with various YE fraction levels (0.5 to 4 g/L). The concentrations of cells and of metabolites were followed throughout cultures.

RESULTS AND DISCUSSION:
The nanofiltration process balance revealed that retentate molecules represented only 26 % of total YE, the majority of YE molecules exhibiting a molecular weight under 300 to 500 Da. Consequently, permeate contained molecules already present in reference medium, plus di-tri peptides and trehalose, while retentate contained molecules lacking in reference medium. Permeate exhibited similar stimulating effect than YE on maximal cell density and IgG production, highlighting the interest of nanofiltration process to refine YE and simplify dowstream processing. Furthermore, permeate reconstitution in amino acids and trehalose underlined that di and tri-peptides was used as source of nitrogenous substrate. On the other hand, the activity of the retentate, which increased the specific cell growth rate, was shown to be mainly due to cationic oligopeptides.

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REF-A078

PROCESS EVALUATION FOR THE PRODUCTION OF A LABILE RECOMBINANT PROTEIN

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KEY WORDS:
PERFUSION PROCESS / CHO CELLS

BACKGROUND AND NOVELTY:
Growing worldwide competition and patent expirations in the biopharmaceutical arena are increasingly turning process productivity into a central issue in the development of animal cell-based technologies. Perfusion is an important tool in this context, because high-cell-density processes operating continuously can be established, which are of advantage for the production of unstable molecules due to the low residence time of the product in the bioreactor. In this work, medium supplementation, operational conditions and two cell retention devices were investigated with the aim of developing an efficient perfusion process for the production of a labile therapeutic protein.

EXPERIMENTAL APPROACH:
A recombinant CHO cell line was cultivated in the customized chemically defined, animal-component-free medium "TC-LECC", derived from a commercial culture media platform (TeutoCell AG). The effect of supplementation of this medium with a recombinant hormone on cell growth and productivity was investigated. Precultures and batch runs were carried out in shake flasks at 37oC, 5% CO2, 180 rpm and 5-cm shaker orbit. Perfusion cultures were carried out in stirred bioreactors at 30% air saturation, and temperature and pH shifts were investigated. Two different cell retention devices (the ATF system and a gravity settler) were evaluated.

RESULTS AND DISCUSSION:
Regarding the hormone supplement, its absence in perfusion led to higher cell density, but less active product. Regarding pH, a shift down to 6.7 resulted in increased product activity, but a decreasing temperature shift did not enhance active product concentration. Among the two different cell retention devices, limitations regarding the retention of the product inside the bioreactor were experienced with the ATF system. The reasons for this are currently under further study. Using the gravity settler, product activity in the bioreactor and in the harvest were comparable, and a process stably operating for over 1 month at high cell concentrations could be established.

REF-A079

THE CHALLENGE TO SCALE UP A STIRRED SINGLE-USE BIOREACTOR FROM 50 TO 2000 L

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KEY WORDS:
SINGLE-USE / BIOREACTOR / SCALE UP / CHARACTERIZATION / QUALITY BY DESIGN

BACKGROUND AND NOVELTY:
Single-use bioreactors are an attractive technology for biopharmaceutical industry. They are excessively used for mammalian cell cultivations e.g. for the expression of vaccines or monoclonal antibodies. This is motivated by several advantages of these bioreactors like reduced risk of cross contaminations or short lead times. Commonly, single-use bioreactors differ in terms of shape, agitation principle and gassing strategy. Hence, a direct process transfer or scale up can be challenging. Consequently, re-usable bioreactors are still regarded as gold standard due to their well-known and defined geometrical properties.

EXPERIMENTAL APPROACH:
Based on this knowledge a stirred single-use bioreactor family was developed with similar geometrical ratios.
To follow a Quality by Design (QbD) approach the single-use bioreactor family evaluated here was characterized by using process engineering methods. For definition of a control space specific power input per volume, mixing times and kLa were determined for scales from 50 to 2000 L.

RESULTS AND DISCUSSION:
The process engineering characterization indicates that these systems are suitable for cultivations of mammalian cells. Based on the data scale up and process transfer are possible between this bioreactor family as well as re-usable systems. Therefore, the presented stirred bioreactors are a major progress for single-use technology.

EVALUATION OF FED BATCH AND PERFUSION CULTURE CONDITIONS FOR PRODUCTION OF A MONOCLONAL ANTIBODY

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KEY WORDS:
CELL CULTURE / FED BATCH / PERFUSION

BACKGROUND AND NOVELTY:
To reduce production costs for new pharmaceutical ingredients speeding up of the process development phase is of importance. Fed batch is the preferred cultivation technique in the pharmaceutical industry. It is simple and robust. However, it has been shown that perfusion cultivation can achieve 5 – 10 times higher productivity. The major weakness of perfusion is its higher technical complexity.

Today, most manufacturers apply platform technologies in Upstream and Downstream processes. However, obtaining high performance requires adaptation of the platform process to different cell lines and/or media. It is therefore advantageous to use flexible equipment and to develop protocols for rapid estimation of cultivation conditions.

EXPERIMENTAL APPROACH:
In our study we used a model CHO cell line producing the antibody G8.8 against Ep-CAM (Epithelial Cell Adhesion Molecule).
For developing a perfusion process we compared five different cell retention systems (SpinFilter, Cell Settler, Centritech Lab III, Biosep and ATF) with regard to achieve high viable cell densities. In addition, we compared the ATF system with two different membranes (2 µm for retention of cells and 50 kDa for retention of cells as well as antibodies).

Beside the feed medium composition itself the most important process conditions for establishing a fed batch process are amount of feed and feed time course. In addition a fed batch process might be improved by lowering the temperature or changing osmolality at a certain time of cultivation. In shake flask cultivations we used Design of Experiments (DoE) to examine feed volume, time of feed start, time of temperature reduction and time of osmolality increase in a single DoE-run.

RESULTS AND DISCUSSION:
The best results were achieved for perfusion culture with the ATF system with cell densities up to 1.3 x 108 cells mL-1. The next best were the Centrifuge and the Cell Settler with cell densities approximately at 3 x 107 cells mL-1. Using BioSep and Spinfilter, cell densities up to 2 x 107 cells mL-1 were obtained.

A bolus feed applied once a day was used for the fed batch shake flask cultivations. Maximum antibody titer was achieved for a feed of approximately 15 mL per day. Time of feed start has almost no influence. Reduction of temperature and increase of osmolality have a negative influence for the used cell line and medium.

DEVELOPING A SCALABLE, HIGH PERFORMANCE BIO-PRODUCTION PROCESS IN MINIMAL TIME

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KEY WORDS:
CELL CULTURE MEDIA / MICROBIOREACTORS / CHEMICALLY DEFINED / CHO / FEED

BACKGROUND AND NOVELTY:
With the tight timelines associated with the biotherapeutic product development and launch process, process development groups are challenged with developing robust, scalable processes in minimal time. Cell culture media and feeding strategies are critical factors, so it is essential to quickly identify a high performance medium that is easily adaptable and scalable. One strategy is to perform media optimization and feed studies to design a process specific for a particular cell line, but this can be costly and time consuming. An alternative approach is to screen commercially available production media and feeds to quickly identify a high performance medium and feed supplement that can be used as part of a platform process. This strategy speeds time to market, which is critical in launching any new product.

EXPERIMENTAL APPROACH:
A series of studies were performed using scale-down models to quickly identify a commercially available base medium that demonstrated high performance across a panel of CHO lines. The evaluation included the use of a microbioreactor system to model the conditions that would be observed in a scaled up bioreactor system. BD Select™ CD CHO Medium 1, a new, novel CHO medium developed specifically for use as a high performance growth and production medium, demonstrated favorable results across the panel of CHO lines. Feed studies were also conducted using the microbioreactor system and the performance of the selected base medium was further increased with the use of the BD Select™ CD CHO Feed Medium A.

RESULTS AND DISCUSSION:
By using predictive scaled down screening methods, a large number of conditions were screened in minimal time using minimal resources. As a result, BD Select™ CD CHO Medium 1 and BD Select™ CD CHO Feed Medium A were quickly identified as high performing candidates for use in a scaled up process.

REF-A082

PERFUSION SEED CULTURES FOR MORE EFFICIENT PRODUCTION BIOREACTOR UTILIZATION

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KEY WORDS:
PERFUSION / SEED TRAIN / PLANT UTILIZATION / VOLUMETRIC PRODUCTIVITY / ATF

BACKGROUND AND NOVELTY:
The production bioreactor is one of the bottlenecks in GMP biologic manufacturing. Traditional fed-batch production processes consist of an unproductive growth phase where cell mass accumulates followed by a more productive stationary phase where the majority of the drug product is generated. That unproductive growth phase lengthens run duration and lowers volumetric productivity, which leads to inefficient production bioreactor utilization and reduces the output rate. We can improve volumetric productivity and debottleneck production bioreactor usage by shifting the growth phase from the production stage into the N-1 seed train stage. Since it is difficult to sustain high cell densities using traditional batch seed train cultures, we propose the use of perfusion in the N-1 seed train bioreactor.

EXPERIMENTAL APPROACH:
Using alternating tangential flow (ATF) technology, we performed perfusion N-1 seed cultures with two different cell lines. Exponential growth was observed throughout the N-1 duration, with Cell Line A reaching 2x its peak fed-batch VCD and Cell Line B reaching 4x its peak fed-batch VCD. Fouling was not observed for either cell line. At the end of perfusion N-1, the cultures were split into high seed fed-batch production cultures.

RESULTS AND DISCUSSION:
Cell Line A's high seed fed-batch production culture tracked the performance of its low seed process, reaching the same harvest titer as the low seed process in 60% of the time. Perfusion N-1 also enabled Cell Line B's high seed fed-batch production culture to reach 2x the VCD of its low seed process, yielding the same titer as the low seed process in 50% of the time. Thus, it is feasible to double the output in the same amount of time.
We have demonstrated proof-of-concept at the bench scale for using perfusion N-1 to inoculate high seed fed-batch production cultures for increasing output and optimizing production bioreactor utilization. Process and media optimizations for perfusion N-1 and high seed production will also be discussed.

REF-A083

USING DESIGN OF EXPERIMENTS TO DEVELOP A BIPHASIC CULTIVATION STRATEGY FOR OPTIMAL PROTEIN EXPRESSION AND PRODUCT QUALITY

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KEY WORDS:
CHO / FC FUSION PROTEIN / BIPHASIC CULTIVATION / DOE

BACKGROUND AND NOVELTY:
In biphasic cultivations the culture conditions are changed to allow maximum recombinant protein expression after accumulating biomass during an initial phase. However, the influence of specific culture parameters and their optimal setpoint are cell line dependent. Additionally their impact on product quality needs to be investigated. In this study a full factorial design was used to evaluate the influence of two key process parameters, culture pH and temperature, on the process performance. A CHO cell line expressing a Fc fusion protein was grown in a biphasic process. A synergistic effect of both process parameters was observed and compared to the previous standard conditions, a drastic improvement of recombinant protein production as well as protein quality was achieved.

EXPERIMENTAL APPROACH:
CHO cells were grown in batch culture. During late exponential phase pH and temperature were shifted according to a full factorial design. The investigated temperature range was 28.5 to 38.5°C and the pH range was 6.75 to 7.05. A central composite design was used and appropriate parameter combinations to cope with the non-linearity of the response were selected.

The cultures were sampled daily to determine cell growth and viability, metabolite concentrations and recombinant protein production. The cultures were terminated once the viability decreased below 70%. At harvest, the product quality was assessed by determining the fraction of aggregated recombinant protein using size exclusion chromatography.

RESULTS AND DISCUSSION:
The parameter shift had a drastic effect on culture performance. Low temperatures reduced cell growth and nutrient consumption, thereby substantially extending the process duration. At the same time the recombinant protein production was stabilized. Depending on the cultivation temperature, an acidic pH reduced protein aggregation. The optimal parameter combination resulted in a 2.5 fold increase of the final product concentration and reduced protein aggregation from 75% to 2%.

HIGHLY EFFICIENT INOCULUM PROPAGATION IN PERFUSION CULTURE USING WAVE BIOREACTOR™ SYSTEMS

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KEY WORDS:
SEED TRAIN / PROCESS INTENSIFICATION / PERFUSION / WAVE BIOREACTOR

BACKGROUND AND NOVELTY:
Inoculum propagation in animal cell culture is typically done in a series of batch cultures with increasing cultivation volume, until a sufficient cell number to seed the production reactor is obtained. Perfusion cultures can be used to obtain high cell concentrations and drastically increase the split ratio. Especially in combination with disposable bioreactors, the turnover time can be shortened and the process flexibilty improved. We developed such a process in a single use WAVE Bioreactor and compared it to traditional batch cultures for inoculum propagation.

EXPERIMENTAL APPROACH:
CHO-S cells were grown in batch and perfusion cultures in single use WAVE Bioreactor systems. In the perfusion process, the cells were retained by a filter integrated into the reactor. The medium renewal rate was increased according to the cell growth. Cells were removed from both cultures in late exponential phase and used to seed fedbatch cultures.

All processes were sampled daily to determine cell growth and viability as well as metabolite concentrations. The fedbatch cultures were terminated once the viability decreased below 60%.

RESULTS AND DISCUSSION:
The maximum cell concentration in batch culture reached 5.1E+06 c/mL while in perfusion a tenfold higher concentration of 4.8E+07 c/mL was achieved. This allowed to increase the split ratio more than 6 fold to about 1:30 for inoculum propagated in perfusion culture. The subsequent fedbatch cultures gave similar results regarding cell growth, viability and cell metabolism.

The method described can reduce the number of expansion steps and eliminate one or two bioreactors in the seed train. Disposable bioreactors at benchtop scale have the potential to directly inoculate volumes of up to 1000 liters. Alternatively, the high biomass concentrations achieved in perfusion culture can be used to seed production bioreactors at increased cell concentrations, thereby shortening the process time in these vessels.

STRATEGY OF RAPID CHO CELL LINE GENERATION FOR THERAPEUTIC ANTIBODY PRODUCTION

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KEY WORDS:
CELL LINE DEVELOPMENT / CHINESE HAMSTER OVARY / ANTIBODY PRODUCTION / DEEPWELL PLATES

BACKGROUND AND NOVELTY:
Development of a platform process for generating Chinese hamster ovary (CHO) cell lines is critical for successful clinical and commercial campaign of target biologics. Although Agensys is a small biotech company, our goal for cell line development remains same as many big pharmaceutical companies. The primary goal of cell line development at Agensys is to create cell lines with high productivity, stable expression, and desirable product quality.

EXPERIMENTAL APPROACH:
We employed ClonePix FL for a primary screening post transfection and also introduced micro-well plates for intermediate screening and expansion. Initially we operated 24 deepwell plates in a batch mode but screening of the clones in the batch mode would ignore the response of clones to the nutrient feeding applied in fed-batch production, therefore, may lead to the miss of the best potential clones. Therefore we developed a feeding strategy for the 24 deepwell plate fed-batch cultures. Recently we also brought in a micro-bioreactor system for the clone evaluation.

RESULTS AND DISCUSSION:
ClonePix enables us to screen more clones after transfection while introduction of the 24 deepwell plates allows us to screen clones in suspension culture at the early stage. Screening of more clones at the early stage increases probability of identifying high antibody producers. Screening of the clones in suspension culture is more predictive than those in static culture. The micro-bioreactor system mimics the culture conditions of large-scale bioreactor and leads us to evaluate more clones in the bioreactor environment. As a result, our cell line development platform process enables us to make a rapid identification of cell lines to be used in clinical and commercial manufacturing.

REF-A086

EVALUATING AND MINIMIZING SEQUENCE VARIANTS DURING RECOMBINANT PROTEIN PRODUCTION

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KEY WORDS:
SEQUENCE VARIANTS / RECOMBINANT

BACKGROUND AND NOVELTY:
Amino acid sequence variants are defined as unintended amino acid sequence changes that contribute to product variation with potential impact to product safety, immunogenicity and efficacy. Therefore, it is important to understand the propensity for sequence variant (SV) formation during the production of recombinant proteins for therapeutic use. Coupling increasingly sensitive analytical techniques with the natural rate of spontaneous mutations and translational infidelity rates in the production of endogenous proteins, it is not surprising to find low levels of sequence variants in recombinant proteins.

EXPERIMENTAL APPROACH:
Experiments to identify strategies to prevent SVs were performed in Chinese Hamster Ovary (CHO) and E. coli production systems in bioreactor scale-down models. Amino acid feeding strategies were employed to prevent misincorporations of specific amino acids that were depleted during the production process. Codon replacement was also utilized to prevent known codon mistranslations.

RESULTS AND DISCUSSION:
This work describes strategies to prevent sequence variant formation during recombinant protein production in CHO and E. coli cells. These strategies include amino acid feeding and codon replacement. Other sequence variants which cannot be mitigated by these strategies may be managed through manufacturing process controls.

REF-A087

ULTRA SCALE-DOWN DISCOVERY OF LOW SHEAR STRESS PROCESSING FOR SELECTIVE RECOVERY OF NEXT GENERATION FUSION PROTEINS

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KEY WORDS:
FUSION PROTEIN / ULTRA SCALE-DOWN / SHEAR STRESS (PRIMARY RECOVERY) / CENTRIFUGATION / FILTRATION

BACKGROUND AND NOVELTY:
Fusion proteins offer the prospect of next generation biopharmaceuticals with multiple functions. We investigated the primary recovery of a novel fusion protein consisting of modified E2 protein from hepatitis C virus fused to human IgG1 Fc and expressed in a Chinese Hamster Ovary cell line. Fusion protein products pose increased challenges in preparation and purification. Issues of concern include the impact of process stress on cell integrity resulting in increased presence of product-related contaminants. This presentation addresses the use of low cost microwell-based ultra scale-down (USD) methods for characterising the integration of cell culture and cell removal operations to develop a bioprocess strategy.

EXPERIMENTAL APPROACH:
All cell culture broth was produced using 5 L stirred bioreactors. USD studies were used to predict removal of contaminants such as lipids, nucleic acids and cell debris as well as fusion protein recovery. A USD shear device was used to mimic the shear stress that occurs in the feed zones of non-hermetic and hydrohermetic centrifuges. For a low shear stress processing alternative, USD depth filtration technique based on the use of a robotic handling platform was used to investigate the filtration performance.

RESULTS AND DISCUSSION:
Based on the results, depth filtration delivered greater solids removal than centrifugation but a small (~10%) decrease in yield of the fusion protein was observed. Both centrifugation and filtration demonstrated little to no cell breakage. USD observations of product recovery and carryover of contaminants were also confirmed at pilot-scale, as was also the capacity or throughput achievable for continuous centrifugation or for depth filtration. The advantages are discussed of operating a lower yield cell culture and a low shear stress recovery process in return for a considerably less challenging purification demand.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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RAPID GENERATION OF CHEMICALLY DEFINED CELL CULTURE MEDIA AND FEED FOR IMPROVED PROCESS CONSISTENCY IN MONOCLONAL ANTIBODY PRODUCTION

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KEY WORDS:
MONOCLONAL ANTIBODY / RAW MATERIALS / PRODUCTION MEDIA / FEED SUPPLEMENT / PROCESS CONSISTENCY

BACKGROUND AND NOVELTY:
Achieving process consistency is a constant challenge for large scale manufacture of biotherapeutics using CHO cell based systems. Cell growth, productivity, and product quality in upstream processing have historically been impacted by the quality of the raw materials used in the manufacture of commercial media and feed supplements. EMD Millipore recently introduced CellventoTM CHO-200, a chemically defined (CD) animal origin free (AOF) dry powder media and companion feed designed for use with CHO cells in fed batch culture.

EXPERIMENTAL APPROACH:
In the following work, we have used a model IgG1 expressing recombinant CHO cell line, and statistical design of experiments (DOE), to formulate a production media and feed supplement with superior purity and performance consistency. Specifically, we will show data comparing growth, productivity, and product quality using multiple lots of Cellvento CHO-200. In addition, we have used a nominal fed batch process to compare the performance of Cellvento CHO-200 against other commercially available media and feeds.

RESULTS AND DISCUSSION:
The results of these studies suggest that Cellvento CHO-200 supports high density cell growth, provides improved production relative to other commercially available media, and is scalable. This work further demonstrates that superior consistency can be achieved for cell growth, titers, and product quality when Cellvento CHO-200 is used with an appropriate fed batch process. In summary, Cellvento CHO-200 provides improved process consistency in fed batch cultures relative to other commercially available media and feed.

GENERATION OF STABLE POLYCLONAL POOLS FOR LARGE-SCALE RAPID PROTEIN EXPRESSION

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KEY WORDS:
PROTEIN EXPRESSION / STABLE POOLS / CHO / POLYCLONAL POOLS

BACKGROUND AND NOVELTY:
The ability to generate suitable amounts of recombinant protein products at scale, but under short time lines, is one of the major limitations in early biopharmaceutical development. Improvements to large scale transient expression methods are one solution, however the limited production lifetime of the cells means that resources (DNA, transfection reagents) can become limiting at scale. Stably transfected cell lines have the ability to continuously produce high levels of protein but typically require long development timelines to identify a high producing, stable clone. Here, we present a process to generate stably transfected polyclonal pools suitable for use in large scale manufacture.

EXPERIMENTAL APPROACH:
The traditional method for generating stable transfectant pools involves transfecting cells with the gene(s) of interest and then splitting the contents of the cuvette into two populations in static T175 culture flasks. In comparison the proposed method divides the transfected cells into 96-well plates. The idea is to isolate transfectant populations to minimise outgrowth of non-expressing cells. The pools are screened using a high-throughput product assay and then the top producing wells are recombined to form a “Superpool.”

RESULTS AND DISCUSSION:
Antibody expression from pools generated using both methods was evaluated using a fed-batch shake flask model. A product concentration of 2 g/L was achieved using the Superpool method compared to 0.4 g/L with the T175 method. The product concentration increased to nearly 3 g/L when the Superpool was generated using the GS Xceed™ cell line. Repeating the fed-batch assessment 50 generations later showed the Superpools in GS Xceed™ maintained high levels of product expression whereas T175 pools and CHOK1SV Superpools lost almost all expression. The increase in the length of the manufacturing window makes this approach an efficient cost-effective solution for early material supply and is capable of producing over 500 g of monoclonal antibody in less than 6 weeks.

REF-A090

EXPANDING THE MOLECULAR TOOLBOX FOR CELL LINE DEVELOPMENT

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KEY WORDS:
VECTOR / XCEED / GS GENE EXPRESSION SYSTEM / CELL LINE / CHO

BACKGROUND AND NOVELTY:
The GS Gene Expression System™ and the new GS Xceed™ Gene Expression system are powerful tools for the generation of cell lines suitable for the production of recombinant therapeutic proteins. The host cells and production processes employed with the system contribute to the ability to rapidly generate highly productive cell lines; however this is underpinned by the vector architecture in the GS vectors themselves.

EXPERIMENTAL APPROACH:
In order to meet the challenge posed by evolving molecular design and the demand for shorter timelines, a flexible toolbox approach for vector generation has been employed which comprises a number of different vector and coding sequence designs. In order to eliminate issues with mRNA structure, coding sequences can be gene-optimised and a choice of signal peptide can also be incorporated into the molecule design. Further modifications have led to the development of GS vectors that are suitable for the production of more complex molecules, such as bispecific antibodies, where the concurrent production of three polypeptides is required. As these vectors contain the ability to produce recombinant protein from a third expression cassette, they also allow for the co-expression of any gene of interest alongside a two-chain therapeutic recombinant protein. Potential applications could be to achieve post-translational modifications not typically possible in the CHO host cell, or to allow for cell line engineering by co-expression of additional protein species.

RESULTS AND DISCUSSION:
The development of the toolbox is not limited to the generation of vectors for non-standard molecules as vector design can also contribute to a reduction in the time taken for cell line development. Robust cloning procedures that enable the generation of vectors with minimal subcloning efforts have been developed that allow for the generation of final expression vectors for monoclonal antibody production in the shortest timeframe.

REF-A091

DISPOSABLE ORBITALLY-SHAKEN TUBESPIN® BIOREACTOR 600 FOR INSECT CELL CULTIVATION IN SUSPENSION

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KEY WORDS:
ORBITAL SHAKING / DISPOSABLE BIOREACTOR / INSECT CELLS / GAS TRANSFER / CO2 STRIPPING

BACKGROUND AND NOVELTY:
Insect cells are a major host for recombinant protein production and are typically grown in suspension culture in spinner flasks or Erlenmeyer shake flasks. As an alternative vessel for medium-scale cultures, disposable conical tubes with ventilated caps having a nominal volume of 600 mL (TubeSpin® bioreactor 600, TPP) have recently been introduced.

EXPERIMENTAL APPROACH:
In this study, we compared cultivation of three widely-used insect cell lines (Sf9, S2, and High-fiveTM) in the TubeSpin® bioreactor 600, spinner flask, and shake flask. We measured cell growth and environmental culture conditions (pH, pO2, pCO2) in batch cultures.

RESULTS AND DISCUSSION:
For all three cell lines, a higher cell density was achieved more rapidly in the TubeSpin® bioreactor 600 than in the other two vessels. In the spinner flask and shake flask, but not in the TubeSpin® bioreactor 600, we observed oxygen limited conditions for all three cell lines under conventional culture conditions. This study validates the TubeSpin® bioreactor 600 for cell culture applications with suspension-adapted insect cells.

IMPROVING ADENOVIRUS PURIFICATION BY USING MEMBRANE CHROMATOGRAPHY. FROM A BIACORE CHIP TO A CONTINUOUS CHROMATOGRAPHY

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KEY WORDS:
MEMBRANE CHROMATOGRAPHY / DOWN SCALE / HIGH THROUGHPUT SCREENING (HTS) / SIMULATING MOVING BED (SMB) / BIACORE

BACKGROUND AND NOVELTY:
Membrane adsorbers are used in the biopharmaceutical industry almost exclusively in flow through mode for the mAb industry. More recently membrane adsorption chromatography has been applied for the purification of viral vectors in a bind elute mode yielding good overall recovery rates. However some issues still remain. For instance, the impact of the ligand density on virus purification, virus adsorption/desorption as well as the effect of competitive binding between impurities such as proteins and DNA with the target virus have to be better understood.

EXPERIMENTAL APPROACH:
In the present study the impact of new ligands (both quaternary and primary ammine), and their density on an anionic exchange membrane were assessed; also matrix structure namely grafted and not grafted were investigated. In addition, the optimal ligand density was selected for implementation of Simulating Moving Bed (SMB) chromatography.

The experiments were conducted by using a Biacore on chip technology for ligand evaluation; subsequently, 96 well plates was used to asses best ligand density and matrix structure suitable for adenovirus purification.

RESULTS AND DISCUSSION:
Our results indicate that increasing in ligand density creates more binding sites for DNA with an increase of 10-fold in the dynamic binding capacity (DBC), but not for viruses or HCP. The membrane with medium ligand concentration showed the best results in terms of purity, and recovery yield for the virus. However the low ligand density was implemented for SMB purpose due to their low irreversible binding. Also, primary amine ligands showed a suitable approach for a novel flow through purification strategy.

This work contributes to the understanding of the physico-chemical interactions between viruses and chromatographic membranes. Furthermore it shows the feasibility of Biacore sensor chip and 96 well plate as High Throughput Screening (HTS) tools. Moreover the selection of suitable membrane of membrane for SMB will pave the way for a robust continuous chromatography.

RAPID GENERATION OF RECOMBINANT PROTEINS BY TRANSIENT GENE EXPRESSION IN HIGH FIVETM INSECT CELLS

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KEY WORDS:
HIGH FIVE CELLS / TRANSIENT GENE EXPRESSION / RECOMBINANT PROTEINS / TNFR-FC

BACKGROUND AND NOVELTY:
Insect cells are frequently used for the production of recombinant proteins following gene delivery with the baculovirus vector- expression system (BVES). However, the BVES has several disadvantages, mainly due to the cytolytic activity of the virus. As an alternative, we established an efficient transient transfection process for rapid recombinant protein production with High FiveTM insect cells using a chemical reagent for transfection. This cell line has been proven to be suitable for the efficient generation of complex, glycosylated proteins using the BVES.

EXPERIMENTAL APPROACH:
Various process parameters, including the culture medium, cell viability, cell density, expression vector, and plasmid DNA and reagent amounts, were optimized.

RESULTS AND DISCUSSION:
With an optimized process, we obtained a transfection efficiency of 90% using enhanced green fluorescent protein (EGFP) as a reporter gene. Transfection with the tumor necrosis factor receptor (TNFR)-Fc fusion protein gene resulted in a volumetric yield of 150 mg/L by 4 days post-transfection. Research is ongoing to reduce the plasmid DNA amount needed for transfection and to scale up the process.

REF-A094

EVALUATION OF PROCESS PARAMETERS IN SHAKE FLASKS FOR MAMMALIAN CELL CULTURE

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KEY WORDS:
MAMMALIAN CELL CULTURE / SHAKE FLASK / PROCESS PARAMETERS / COMPARABILITY

BACKGROUND AND NOVELTY:
Shake flask cultivation is nowadays a routine technique during process development for mammalian cell lines. Here unbaffled and baffled shake flasks are applied. During shaken culture, changes in agitation velocity, shaking diameter or shake flask size affect the hydrodynamics in the shake flask. This might be reflected in the growth of the cultured cells.

Process parameters such as power input, mixing time, fluid velocity etc. have been determined and described mathematically for shake flasks used for microbial cultivation, but only to some extend for mammalian cell culture. Especially the relationship between these parameters and growth characteristics of mammalian cells is still a relatively uncovered issue.

EXPERIMENTAL APPROACH:
In this work, process parameters like specific power input, mixing time, maximum fluid velocity and Reynolds number were determined for four different shake flasks in a range of shaking velocities on a shaking machine. The specific growth rate (μmax) of the human production cell line AGE1.HN® (ProBioGen AG, Berlin, Germany) was compared to the respective process parameters.

RESULTS AND DISCUSSION:
Our results point to regions of the studied parameters, where common operation windows can be identified for μmax. In these process windows the cells show a similar μmax in different shake flask, making cell growth comparable. These process windows are common for the flasks, independently of their size and their number of baffles.

The data obtained in this work can be used for process standardization and comparability of results obtained in shaken systems i.e. to guarantee consistency of results generated during research tasks using mammalian cells.

REF-A096

SCALE-UP PRODUCTION OF PHARMACEUTICAL PROTEINS IN PLANT CELL SUSPENSIONS WITH ORBITALLY SHAKEN DISPOSABLE BIOREACTORS

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KEY WORDS:
BY-2 / CELL CULTIVATION / RECOMBINANT ANTIBODIES / SINGLE-USE BIOREACTORS / TOBACCO

BACKGROUND AND NOVELTY:
Plant cells are well suited for the production of pharmaceutical and industrial proteins either as whole-plant systems or cell suspension cultures. The latter have the advantage of being cultivated in containment under defined conditions that allow rigid process control. Cylindrical orbitally shaken single-use bioreactors are potentially favourable types of cultivation vessels for plant suspension cells because they combine reduced cell stress and contamination risk, that are characteristic for surface aerated reactors, with the flexibility and cost efficiency of disposables.

EXPERIMENTAL APPROACH:
We investigated the suitability of 50-mL TubeSpin Bioreactors, 10-L to 50-L Nalgene vessels and the 200-L OrbShake (SB-200X, Kuhner) orbital shaker for the cultivation of transgenic tobacco BY-2 cells secreting a human IgG antibody to the medium. For all scales, the oxygen consumption of the cells was online monitored during cultivation. A downstream process strategy for efficient antibody capture from the spent medium was developed and the production process including aspects of scalability, downstream processing and production costs was evaluated.

RESULTS AND DISCUSSION:
Aiming at the demonstration of process scalability, antibody producing BY-2 suspension cells were cultivated in cylindrical orbitally shaken bioreactors with nominal working volumes ranging from 5 mL to 100 L. The biomass accumulation and product formation obtained during cultivation in the 200-L bioreactor matched the performance of shake flask cultured cells. Thus, a 20-fold scale-up in culture volume did not adversely affect the productivity of the plant cells. The dissolved oxygen tension was measured online as a reliable indicator for cell growth. After 130-h cultivation almost one gram of antibody was harvested from the spent medium and purified resulting in a final product recovery of 90%. Our results prove the suitability of orbitally shaken bioreactors for the scale-up production of pharmaceutical proteins in tobacco cell suspensions.

A GFP-BASED APPROACH FOR THE OVEREXPRESSION AND PURIFICATION OF MAMMALIAN MEMBRANE PROTEINS

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KEY WORDS:
MEMBRANE PROTEINS / PROTEIN PRODUCTION / PROTEIN PURIFICATION / TAO2 / GFP FUSION

BACKGROUND AND NOVELTY:
Production and purification of integral membrane proteins (IMP) for structural studies is difficult mostly due to the very low yields obtained and to protein instability. To date fewer than 300 structures of IMPs are known, less than 0.5% of all the known structures. For eukaryotes the story is even starker, with less than 50 mammalian IMPs solved. Membrane protein production and purification is therefore one of the most important remaining frontiers for structural biology research. One way to improve the throughput of eukaryotic IMP structures is to develop methods that reliably facilitate the identification of well expressing constructs that are stable and functional. In this work, we used the multi-spanning membrane MAP3K kinase TAO2 as a model to setup a GFP-based approach for the fast and cost effective multi-host expression screening towards the purification of eukaryotic IMP.

EXPERIMENTAL APPROACH:
TAO2 was cloned fused to GFP and a polyhistine tag into pOPIN-F, a vector designed to enable high-level target gene expression in multiple systems (1). A GFP-based expression screening and optimization was carried out using different E. coli strains, mammalian and insect cells to determine the host best suited for TAO2 production. GFP fluorescence was further used to facilitate detergent screening for downstream processing.

RESULTS AND DISCUSSION:
GFP was found to be a fast measure ofor protein expression and localization. By monitoring GFP fluorescence, we show that the TAO2 fusion protein only localizes correctly throughout the cell membrane when expressed in mammalian cells and only when GFP is present at the N-terminus. We indicate the practical steps that constitute our GFP-based pipeline for expression evaluation and to speed up membrane extraction detergent screening for protein purification. In short, although membrane-integrated expression is no guarantee of function, the GFP-tag speeds the empirical process towards obtaining stable and homogeneous material for functional and structural work.

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IMPROVEMENT OF CELL-FREEZING TECHNOLOGIES AND DISPOSABLE BIOREACTORS: TOWARD A FULLY CLOSED USP

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KEY WORDS:
DISPOSABLE / BIOREACTOR / CLOSED / COMPARABILITY

BACKGROUND AND NOVELTY:
Biotech processes still contain a number of open and at risk transfers steps. Cell culture steps from one frozen vial to containers such as shake flasks is one of these open phases. This critical expansion step may take several days or weeks and delay development or production timeline

due to contamination. By coupling traditional technologies and new technologies (disposable bags, cases and bioreactor), a study on bags freezing conditions and scalability of single-use bioreactors (SUB) has been performed in order to define a fully closed USP process.

EXPERIMENTAL APPROACH:
We have evaluated the freezing/thawing of cells in bags for fully closed operations from thawing to 1250L Bioreactor inoculation. A first trial on 7 different CHO cell lines was performed and demonstrated the feasibility of this approach. With this technique, cell amplification timelines were reduced and the risk of cross contamination eliminated. This first improvement was combined to SUB that are now commonly used for process development and as seeding or production bioreactors. If the benefits associated to these equipments have been well demonstrated on more than a decade, only a few data on their scalability are published.

During the period 2010-2012, we performed a study in order to evaluate the performances of SUB at various scale from 3L to 200L. The evaluation was performed both for seeding application and for clinical material production. Several clinical runs at 200L and 1250L scale were performed to ensure a meaningful comparison. These performances were also compared to glass and stainless steel bioreactors of different sizes ranging from 3.6L and 1250L.

RESULTS AND DISCUSSION:
Coupling cell freezing in bag and disposable bioreactors up to production scale allowed us to develop a fully closed USP process. We will extensively discuss the final set up from a technical, financial and organizational point of view emphasizing the various savings associated (labour, expense, training…) with single use systems and closed processes.

REF-A099

HOW TO SUCCESSFULLY TRANSFER A STATE OF THE ART CHO FED BATCH PROCESS TO A VARIETY OF SINGLE-USE BIOREACTORS

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KEY WORDS:
SINGLE-USE / BIOREACTOR / CHO / FED BATCH / PROCESS TRANSFER

BACKGROUND AND NOVELTY:
Nowadays, single use bioreactors are widely accepted in pharmaceutical industry. This is based on shorter batch to batch times, a reduced cleaning effort and a significantly lower risk of cross contaminations. The usage of single-use bioreactors for seed-train cultures in mammalian cell culture processes is a common practice. However, now the focus is extended to perform state of the art fed batch production processes in such bioreactors. In this study an industrial proven CHO fed batch process is transferred to different single-use and re-usable bioreactors.

EXPERIMENTAL APPROACH:
To follow a Quality by Design (QbD) approach all cell culture bioreactors evaluated here, which differ in terms of agitation and gassing principle, were characterized by using process engineering methods. For definition of a control space the mixing times and kLa-values were used for each bioreactor type. Based on the results the process parameters of the selected cell culture process were adapted.

RESULTS AND DISCUSSION:
For the verification a model cell line (CHO DG44) was used, which expresses a human IgG in a 17-day fed batch process. After 3 days of batch phase a daily, discontinuous and fully automated feeding procedure was initialized. A peak viable cell density exceeding 20 x 106 cells/mL and a final product titer of more than 5 g/L were achieved in single-use and re-usable bioreactors, respectively. Furthermore, these results are comparable to data from significantly larger scales. The successful process transfer indicates that single use systems are an attractive alternative for state of the art cell culture applications.

REF-A100

FAST TO CLINIC WITHOUT THE NEED FOR CELL LINE SWITCH LATER: USING A HIGHLY PRODUCTIVE PROCESS PLATFORM AND SINGLE-USE REACTOR EQUIPMENT

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KEY WORDS:
HIGH PRODUCTIVITY / FAST TIME LINE / SINGLE-USE REACTORS

BACKGROUND AND NOVELTY:
The need to fast into the clinic and lengthy process development for a particular cell clone has led to the emergence of two cycle development approaches in the past. The first cycle focused on fast to clinic with a process and often a cell line that is not capable of supplying a potential market. A second development cycle and often cell line was required before going to process validation and pivotal clinical testing. This approach resulted in huge cost for cycle 2 development and came with significant comparability risks.

EXPERIMENTAL APPROACH:
Development of potent fed-batch chemically defined process platform.
Establishment of single-use reactor equipment that is capable or executing CD process platform.
Alignment with cell line development that feeds well into platform.

RESULTS AND DISCUSSION:
The development and implementation of a highly productive process platform for a CHO based expression system has enabled us to skip any process development for the initial clinical manufacturing process while still getting acceptable titers in the 5 g/L range for antibodies. The main elements of the CD process included a new media and feed formulation, an improved feeding strategy, the back integration of media and feed materials into cell line development, the development of a high density banking process, establishment of 1000L single use reactor systems and their characterization and finally implementation in a GMP environment. This strategy enables tox material supply within a few months after cell line selection. At the same time the process features high productivity, excellent raw material control and no cell line switch is required for late stage or commercial supply. The presentation will highlight the impact of the different elements of the new strategy and will discuss potential further optimization for even faster transitions.

CHINESE HAMSTER DRAFT GENOME SEQUENCED FROM SORTED CHROMOSOMES

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BACKGROUND AND NOVELTY:

Chinese hamster ovary (CHO) cells are especially prone to chromosomal rearrangements, which causes their plasticity and ease of adaptation, but also their instability. With reduced sequencing costs, it is now feasible to study the occurrence and effect of these rearrangements in more detail. However, this requires a well characterized reference genome with defined chromosomal allocation of sequences. Current short read sequencing technology alone does not allow such assignments and is likely to miss the precise location of translocations or duplications.

EXPERIMENTAL APPROACH:

Chromosomes of inbred Chinese hamsters were sorted by flow cytometry and sequenced in separate sequencing reactions. This was complemented by mate-pair sequencing of full genome DNA for improved assembly and scaffold construction. Sorting of the chromosomes is a challenging task. Therefore, the separated chromosome assemblies were filtered to eliminate unavoidable contaminations from the single zzassemblies.

RESULTS AND DISCUSSION:

A draft genome of Cricetulus griseus is presented, consisting of scaffolds and contigs for chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9+10 (not distinguishable by FACS), and X. Except for mouse chromosome 7, which is insufficiently covered, the rest of the mouse genome is highly covered by Cricetulus griseus sequences. Gaps occur primarily at regions with a high frequency of interspersed repeats and low complexity. CHO-K1 contains almost the entire Chinese hamster genome: gaps relative to the mouse occur at the same positions of high repeat density and only very small regions are completely missing. It cannot be differentiated at present whether these are indeed missing or could not be properly assembled. The results provide a reference genome for future CHO research of sufficient quality and precision to enable detailed studies of chromosomal rearrangements.

OTHER INFORMATION

Keywords: Chinese hamster Cricetulus griseus genome, chromosome sorting.

TRANSIENT TRANSFECTION OF HIGH-DENSITY CULTURES OF SF9 CELLS FOR IMPROVED RECOMBINANT PROTEIN YIELDS

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KEY WORDS:

SF9 CELLS / HIGH CELL DENSITY / RECOMBINANT PROTEIN / TRANSIENT GENE EXPRESSION

BACKGROUND AND NOVELTY:

Insect cells have been widely used for the production of recombinant proteins, using recombinant baculovirus for gene delivery.

EXPERIMENTAL APPROACH:

To simplify protein production in insect cells, we developed a method based on transient gene expression (TGE) with cultures of suspension-adapted Sf9 cells using a chemical reagent for DNA delivery. The transfection medium, both the plasmid DNA/ reagent amounts, and the cell density were optimized.

RESULTS AND DISCUSSION:

Transfection at high density improved the volumetric yield to over 100 mg/L for a secreted protein in a 4-day process, as compared to cells transfected at low density. The increase in yield was not due to an increased cell number since each transfected culture was diluted to the same density (4 x 106/mL) and volume after transfection. High-density transfection resulted in a significant percentage of cells being blocked in the G2/M phase of the cell cycle for the first 24 h post-transfection. Thereafter, cells divided normally and obtained the same maximal density as cells transfected at low density. The high-density TGE method was found to be scalable from 10 to 300 mL.

REF-A103

STREAMLINED PROCESS DEVELOPMENT USING THE MICRO 24 BIOREACTOR SYSTEM

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KEY WORDS:
MICRO24 / PROCESS DEVELOPMENT / SCALE DOWN / MICROBIO-REACTORS

BACKGROUND AND NOVELTY:
Increasing time and cost pressures over the past few years have helped to accelerate the development of microbioreactors (MBR) and their integration into the development of therapeutic protein production processes. Several systems have been commercialised and are now routinely used for cell line screening and process development applications where the ability to complete meaningful process optimisation experiments in scale down systems can have significant cost, resource and time implications. In this study we describe the use of the Micro24 Bioreactor system to carry out process development experiments using a Design of Experiments approach to identify process critical parameters and the subsequent validation of these results in bioreactors.

EXPERIMENTAL APPROACH:
Lead antibody-producing CHO cell lines were selected using shake flask models of basic platform production processes and in order to 'fine tune' the basic processes for specific cell lines the effects of a number of potentially process relevant factors including pH and dissolved oxygen were then assessed in a Micro24 Bioreactor system. Modified process conditions leading to improved cell line performance were then confirmed in conventional bioreactors.

RESULTS AND DISCUSSION:
Cell number, titre, specific productivity and product quality profiles obtained from DoE experiments in the Micro24 Bioreactor system were used to identify those factors having the greatest effect on overall process performance. These were confirmed in bioreactors where the modified processes resulted in increased titres and/or specific productivities compared to the original 'control' process.

These results have shown that process improvements identified at the sub-10mL scale in the Micro24 are translatable to conventional bioreactors and therefore demonstrate the utility of the Micro24 Bioreactor system for scale down mammalian process improvement.

REF-A104

SCALE-UP OF VERY HIGH CELL DENSITY CHO CULTURES (ABOVE 130 MILLION CELLS PER ML) IN SINGLE USE STIRRED-TANK BIOREACTORS AT 200 L SCALE AND BEYOND USING XD® CULTURE

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KEY WORDS:
SINGLE USE BIOREACTOR / SCALE-UP / VERY HIGH CELL DENSITY

BACKGROUND AND NOVELTY:
XD® culture is a proprietary DSM technology that is characterized by short processes with extremely high viable cell densities at high viabilities. Routinely around 150 million cells per mL can be obtained in a 2 week process with CHO and other cell lines. Due to the resulting high IVCs, typically a 5 to 10 fold titer boost is achieved compared to classical Fed-Batch operation with the same process length. This enables the use of relatively small (single use) bioreactors, typically 500 – 1000 L scale, for commercial scale production. This in turn enables substantially lower cost production plants with commercial scale output.

In this work the successfull XD® cell cultures in large scale (200-500 L) disposable bioreactors achieving cell densities exceeding 130 million cell per mL and the work leading up to these results are presented.

EXPERIMENTAL APPROACH:
The scale-up strategy of the XD® technology to 200 and 500 L scale in single use bioreactors consisted of a/ definition of the process requirements for the XD® process b/ theoretical assessment of the available single use bioreactors on the market for XD® is presented c/ selection of the most suitable disposable bioreactor and testing of kla with different sparger and impeller configurations d/ performing a 200 L scale bioreactor pilot cell culture experiment e/ installing and qualifying of a 500 L scale bioreactor.

RESULTS AND DISCUSSION:
In this paper, the results of the theoretical bioreactor assessment is presented, as well as the physical characterization of the selected Sartorius Cultibag STR bioreactors. Furthermore the results of the successful 200 L pilot large-scale XD® cell culture, including the process control performance are presented and compared with the results of the 2 L downscaled model.

REC. ST6GAL-I VARIANTS TO CONTROL ENZYMATIC ACTIVITY IN PROCESSES OF IN VITRO GLYCOENGINEERING

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KEY WORDS:
BETA-GALACTOSIDE ALPHA-2,6 SIALYLTRANSFERASE 1 / N-GLYCOSYLATION / GLYCOENGINEERING / TRANSIENT GENE EXPRESSION

BACKGROUND AND NOVELTY:
Glycosylation is an important posttranslational modification of proteins influencing protein folding, stability and regulation of the biological activity. The sialyl mojety (sialic acid, 5-N-acetylneuramic acid) is usually exposed at the terminal position of N-glycosylation and therefore, a major contributor to biological recognition and ligand function, e.g. Fc R-mediated effector function of therapeutic antibodies. In addition, IgG featuring terminal sialic acids were shown to induce less inflammatory response and increased serum half-life.
The biosynthesis of sialyl conjugates is controlled by a set of sugar-active enzymes including sialyltransferases which are classified as ST3, ST6 and ST8 based on the hydroxyl position of the glycosyl acceptor the Neu5Ac is transferred to (Weijers et al. (2008). The ST6 family consists of 2 subfamilies, ST6Gal and ST6GalNAc. ST6Gal catalyzes the transfer of Neu5Ac residues to the hydroxyl group in C6 of a terminal galactose residue of type 2 disaccharide (Gal 1-4GlcNAc).

EXPERIMENTAL APPROACH:
The use of glycosyltransferases for enzymatic synthesis of defined glycan structures will become an essential tool to direct N-glycosylation of therapeutic proteins such as antibodies. Since glycosyltransferases of prokaryotic origin usually do not act on complex glycoprotein structures, sialyltransferases of mammalian origin are preferred. For example, Barb et al. (2009) prepared highly potent sialylated forms of the Fc fragment of immunoglobulin G using isolated human ST6Gal-I. To our knowledge, the access to recombinant ST6Gal-I for therapeutic applications is still limited due to low expression and/or poor activity in various hosts (Pichia pastoris, Spodoptera frugiperda, and E. coli).

RESULTS AND DISCUSSION:
The present study describes the high-yield expression of two variants of human beta-galactoside alpha-2,6 sialyltransferase 1 (ST6Gal-I, EC 2.4.99.1, P15907) by transient gene expression in HEK293 cells with yields up to 100 mg/L featuring distinct sialylation activity.

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INNOVATIONS IN BIOREACTOR DESIGN: HOMOGENEOUS MIXING WITH A SINGLE IMPELLER AND A FLEXIBLE FILM BAFFLE

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KEY WORDS:
MIXING / BAFFLE / BIOREACTOR / SHEAR / IMPELLER

BACKGROUND AND NOVELTY:
Effective mixing is critical for optimization of the bioreactor process. A mixing system provides three basic functions; environmental stability (pH, nutrients, temperature) within the full volume, dispersion of gases for supplying O2 and extracting CO2, and most efficient heat transfer. Providing good mixing, without adding damaging shear effects, is more challenging as the scale of the bioreactor increases. Some commercial systems include one bottom-mounted impeller. The formation of a vortex, with stagnant zones, is often linked to this single agitator. A well designed baffle can be added to suppress vortex formation and provide movement of fluid into preferred patterns of axial and radial flow for homogeneous mixing.

EXPERIMENTAL APPROACH:
From benchtop to process development and clinical scale, with aspect ratios from 0.2:1 to 2:1, colorimetric mixing studies show which features of impellers and baffles are key to providing good mixing across all scales. By using a Phenolphthalein indicator, it is possible to observe mixing patterns as additions of acid and base change the pH of the liquid in the vessels. Mixing time is defined as the time for complete color change throughout the entire volume.

RESULTS AND DISCUSSION:
A unique flexible film baffle was developed to work with a single impeller, to limit shear effects. This baffle has a novel configuration which disrupts vortex formation across the entire vessel height and provides homogeneous mixing for all operating volumes. Results highlight the critical design parameters of the baffle, including geometry, size, and position relative to vortex formation. Short mixing times with uniform dispersion are achieved at the challenging points of 0.2:1 aspect ratio and volumes up to 2000L. Ultimately, proper design and implementation of the impeller/baffle combination provides a mixing solution across a wide range of volumes and aspect ratios, enabling the development of a family of bioreactor systems with excellent scalability and well defined performance.

REF-A107

TOWARD IMPROVED UNDERSTANDING OF CELL DAMAGE IN SPARGER REGION

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KEY WORDS:
CELL DAMAGE / HYDRODYNAMIC FORCES / SPARGER / MODELING / BIOREACTOR

BACKGROUND AND NOVELTY:
To date, most studies focus on cell damage by hydrodynamic forces in bubble breakup region. Among various parameters, energy dissipation rate (EDR) is commonly used to correlate with cell damage. But in recent years, it has also been reported that significant cell damage can occur at the sparger region, especially at high gas entrance velocities (GEV) and with sensitive cell lines. However, there is a lack of good understanding about the mechanism causing cell damage.

EXPERIMENTAL APPROACH:
A case study will be presented to show a clear effect of GEV on cell growth and viability at pilot scale. Furthermore, a Second-Order-Moment (SOM) bubble-liquid two-phase turbulent model based on the two-fluid continuum approach is developed to characterize the turbulent hydrodynamics in sparger region. The model fully considers the anisotropicity of two-phase stresses and bubble-liquid interactions. Using the model, distributions of key hydrodynamic parameters within the bioreactor, such as liquid/bubble velocities, stresses, stress correlations, turbulent kinetic energy, turbulent EDR are calculated.

RESULTS AND DISCUSSION:
It is interesting to find that both stress and EDR data are low in the sparger region compared to bulk, which can't explain cell damage. A new parameter (Stress-induced-Turbulent-Energy-Production, STEP) is proposed, which shows a good correlation with GEV and cell damage. To our knowledge, this is the first time a mathematical interpretation and theoretical analysis of hydrodynamics in the sparger region as well as correlation with cell damage is performed. Some interesting bubble formation patterns captured by high speed camera will be also shared.

REF-A108

DEVELOPMENT AND IMPLEMENTATION OF A GLOBAL ROCHE CELL CULTURE PLATFORM FOR PRODUCTION OF MONOCLONAL ANTIBODIES

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KEY WORDS:
CELL / CULTURE / PLATFORM / CHO / ANTIBODY

BACKGROUND AND NOVELTY:
For the production of recombinant glycoprotein therapeutics (mainly monoclonal antibodies) in mammalian cells, a global Roche cell culture platform was developed. The use of platform manufacturing processes for the production of clinical material has several advantages including lower cost for process development and faster generation of clinical material, thus enabling a reduced timeline to entry into clinical studies.

EXPERIMENTAL APPROACH:
Two independent legacy platform processes of different organizations have been evaluated with the result that some components were merged into the new single platform and other components were jointly developed (e.g. production media and feed). Significant differences in process details which included the use of different (a) CHO host cell lines, (b) proprietary inhouse chemically-defined media and feed formulations, and (c) different bioreactor processes have been overcome.

RESULTS AND DISCUSSION:
One single upstream platform at both Roche process development sites facilitates increased flexibility with regard to process development and manufacturing: any process could be executed at any network facility without significant limitations (plant fit, raw material availability). Another benefit is that all future development efforts could be leveraged by the entire organization leading to more efficient use of resources (e.g., process characterization and validation).

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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POOR RECOVERY FROM THAW TROUBLESHOOTING

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KEY WORDS:
CELL THAW RECOVERY / SEED TRAIN SLOW GROWTH / TROUBLESHOOTING / PH

BACKGROUND AND NOVELTY:
While starting cell processing from a cell bank thaw step into bioreactors using standard procedures for a CHO cell line, challenges were encountered where multiple seed trains had to be discontinued due to low viability and slow growth within the first few passages.

EXPERIMENTAL APPROACH:
Initial scale-down troubleshooting experiments conducted using a shake flask system showed rapid recovery in both viability and growth rates, as opposed to their 2L bioreactor counterparts. Follow-up studies were conducted to examine differences in the two small-scale systems that would result in different thaw recovery rates, including pH control and levels of agitation and aeration.

RESULTS AND DISCUSSION:
Among the factors tested, higher physical stress forces (shear due to agitation/aeration) and cell protectant (pluronic F68) concentration did not have significant impact on recovery from thaw, as measured by cell growth and viability. However, a controlled pH study conducted in 2L bioreactors over a range of pH setpoints showed higher bioreactor pH setpoint yielding better performance in the thaw and early seed train passages. Based on these findings, thaw performance characterization studies may be useful to conduct on specific cell lines to better understand the optimal operating ranges for cell line thaw and early seed train.

A SYSTEMATIC STRATEGY OF CELL CULTURE MEDIA AND FEED DEVELOPMENT

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KEY WORDS:
DOE / MEDIA / MEDIA PLATFORM / MEDIA COMPONENT DOE / PRODUCT QUALITY

BACKGROUND AND NOVELTY:
Development of a high-performance and robust fed-batch process for recombinant Chinese Hamster Ovary (CHO) cell lines presents challenges in light of the diverse nutritional requirements observed with different clonally derived cell lines as well as the varied production phases. To address these challenges, we will discuss an innovative strategy of media, feed, and process development that has been developed at FUJIFILM Diosynth Biotechnologies (FDB). The efficiency and effectiveness of this strategy have been well demonstrated in multiple mAb-producing CHO cell lines at varied scales, with a good balance between product yield and quality.

EXPERIMENTAL APPROACH:
A Design of Experiment (DOE) based developmental approach has been implemented throughout this strategy, which is further segregated into a number of sub-categories; including media and feed “tool box” development, media platform development, media component DOE and their impact on titer and quality. The cell culture performance has also been confirmed at varied scales from shake flask, 2 L bioreactor, and 200 L bioreactor to indicate acceptable process scalability.

RESULTS AND DISCUSSION:
FDB has established a systematic strategy of cell culture media and feed development to fit diverse nutritional requirements of varied-origin CHO cell lines. The value of this strategy is not only to improve cell culture performance with shortened development duration (i.e. 5-fold increases in IgG titer after using the media “tool box” in a case study), but also to help develop manufacturing-friendly processes through simplifying the feed strategy. In addition, feed components have been linked to product quality (e.g. glycosylation) by using media component DOE study, which further strengthens the effectiveness of this development strategy. These combined results provide tremendous flexibility for optimization of culture productivity and product quality while also providing a simple and well defined manufacturing process.

REF-A111

PROTEIN FOLDING AND GLYCOSYLATION PROCESS ARE INFLUENCED BY MILD HYPOTHERMIA IN BATCH CULTURE AND BY SPECIFIC GROWTH RATE IN CONTINUOUS CULTURES OF CHO CELLS PRODUCING RHT-PA

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KEY WORDS:
CHO CELLS / CONTINUOS CULTURES / TEMPERATURE / PROTEIN FOLDING / GLYCOSYLATION

BACKGROUND AND NOVELTY:
CHO cells are the main host for producing recombinant proteins for human therapeutic use, because their capability to make properly folding and glycosylation processes. However, this expression system still present low productive capacity and different approachs are used to improve their performance, one of the most important and promissory is the utilization of mild hypothermia, but their effects on protein folding and glycosylation are poorly understood.

EXPERIMENTAL APPROACH:
To improve understanding about this phenomena, our work group investigated, in two kind of cultivation systems (batch and continuous culture), the effect of temperature (33°C and 37°C) on protein folding and glycosylation processes, using inhibitors for the processes of translation, glycosylation and Endoplasmatic Reticulum Associated degradation (ERAD) pathways I (Ubiquitin/Proteasome way) and II (Autophagosoma/Lisosomal way).

Cell growth was measured by counting cells by trypan blue method; consumption and production of metabolites were measured by biochemical analyzer (YSI 2700); protein rht-PA was measured by ELISA (Trinilize tPA antigen) and enzymatic activity of the protein was measured by amidolytic assay (S-2288 peptide, Chromogenix Italy). The results were analyzed by the mathematical technique of PCA (Principal Component Analysis).

RESULTS AND DISCUSSION:
Results from batch cultures indicate that glycosylation process is sensitive to mild hypothermia, promoting accumulation of intracellular deglycosylated rht-PA under inhibition of glycosylation and ERAD pathways I and II. PCA analysis showed that the general behavior of the cells is modulated by the temperature used. Results from continuous cultures indicate that intracellular rht-PA degradation is promoted by the decrease on specific growth rate rather the use of low temperature. In the same way, the behavior showed by cells is mainly due to lower specific growth rate, as suggested by PCA analysis.

REF-A112

PIGGYBAC TRANSPOSITION FOR THE GENERATION OF STABLE CHO-DG44 CELL POOLS EXPRESSING MULTIPLE TRANSGENES

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KEY WORDS:
STABLE POOLS / PIGGYBAC TRANSPOSON

BACKGROUND AND NOVELTY:
DNA transposons are natural genetic elements that have been engineered to function as gene delivery vectors in mammalian cells. Here, the piggyBac (PB) transposon system was used for the simultaneous delivery of multiple genes into CHO-DG44 cells to obtain recombinant cell pools co-expressing multiple proteins.

EXPERIMENTAL APPROACH:
Four artificial transposons were constructed, each bearing a single transgene and the puromycin resistance gene. Enhanced green fluorescent protein (eGFP), secreted alkaline phosphatase (SEAP), and the light and heavy chains of an IgG1 antibody were used as the four model proteins. Studies were conducted to create stable cell pools expressing different combinations of the four transgenes under various selection regimes. The volumetric productivity, production stability, and average integrated gene copy number of each transgene were determined for each pool.

RESULTS AND DISCUSSION:
The results showed that pools expressing all four model proteins could be generated. However, the productivity of the pools transfected along with the gene for the PB transposase (PBase) were 3 to 10-fold higher in comparison to those generated in the absence of PBase. The volumetric productivity of each individual protein was observed to decrease with an increase in the total number of different transgenes transfected. The reduced integrated copy number of the transgenes in the pools transfected with multiple transgenes could explain, at least in part, the decrease in productivity. The antibiotic concentration or selection duration did not have much impact on the volumetric productivity of the pools. The efficiency of the PB system to co-integrate a higher number of independent transgenes is being investigated by using plasmids bearing different selection markers in order to further improve the stringency of selection. Our data suggest that the PB transposon system provides an efficient method for the co-integration of multiple genes, making it attractive for the stable expression of multiprotein complexes.

REF-A113

WHY BOTHER TO REGULATE THE PH IN MY PRODUCTION BIOREACTOR?

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KEY WORDS:
FED-BATCH / PH / BIOREACTOR / PROCESS OPTIMIZATION

BACKGROUND AND NOVELTY:
In the last decade, monoclonal antibodies have become the most important class of biopharmaceutical drugs. Due to the large dose to be injected to the patients (around 1 g), their production must rely on efficient cell culture processes able to deliver several grams of antibody per liter and scalable up to several thousand liters. Fed-batch processes in bioreactors using mammalian cell lines are the most widely used production system. Despite increased knowledge about optimal cell culture medium composition, cell metabolism, and efficient cell line engineering, the key factors impacting the process performance are still dependent on the cell line used and the cell culture conditions, and the optimization of the process productivity is still a complex exercise.

EXPERIMENTAL APPROACH:
Here we describe the optimization of a fed-batch production process for a fusion protein in NS/0 cell line. Several series of up to eight 5-L bioreactors were run in parallel with different combinations of operating parameters using experimental design. The optimization work was mainly focused on the composition of the supplements added during the culture in order to maintain the cell culture viability and sustain the production of the protein. Metabolite and amino-acid analysis was performed during the culture in order to identify the components of the media becoming limiting for the cells, and this information was used to design the feeds.

RESULTS AND DISCUSSION:
The original process showed a large variability in terms of viability drop and subsequently process duration. In parallel to the feed optimization, several elements pointed out pH regulation as a factor of interest impacting process performance. The implementation of a wide pH range, close to the unconstrained evolution of the cell culture pH, allowed stabilizing the process at a high titer. Further improvement was obtained through the implementation of three feeds. Altogether, the productivity of the process was increased two-fold, leading to a final titer close to 2 g/L.

REF-A114

USE OF PH MANAGING HYDROGELS FOR CLONE SCREENING IN SHAKE FLASKS

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KEY WORDS:
HIGH THROUGHPUT SCREENING / PH / SHAKE FLASK

BACKGROUND AND NOVELTY:
Initial screening of clones during cell line development for recombinant protein production is widely carried out in batch mode without pH control in platforms like shake flasks, whereas production is invariably carried out with nutrient feeding and pH control. This disparity could result in sub-optimal clone selection, and can be addressed with the use of microbioreactors. We will describe the use of pH managing hydrogels (pHmH) in shake flasks as a low cost solution. A mathematical model is developed to understand the effect of pHmH addition on the outcome of clone selection.

EXPERIMENTAL APPROACH:
A suspension CHO cell line is used to experimentally evaluate the effect of pHmH addition on culture pH. The mathematical model incorporates effect of pH on cell growth and cellular metabolism related parameters, and simulates clonal variability. All clones are evaluated in batch and fed batch mode (a) with pH control (b) with addition of pHmH (c) without pH control. The ranking of clones relative to their rank in cultures simulated in fed-batch mode with pH control is used as a measure of the suitability of pHmH application.

RESULTS AND DISCUSSION:
pHmH addition resulted in control of pH between 6.8 to 7.2 over 12 days of culture whereas pH of the uncontrolled culture decreased to 6.4. Clonal variability was simulated by varying cell growth, metabolism and specific productivity parameters. On comparing the clone rankings under different culture methods, simulations confirm that clone ranking under batch conditions, with or without pH control, and interestingly also under fed batch conditions without pH control, is not predictive of clone ranking under pH controlled fed batch conditions. The model predicts that the use of pHmH can increase the utility of shake flasks during clone screening as the ranking of clones generated through this process is better predictive of the ranking under pH controlled fed-batch conditions as compared to screening with uncontrolled pH.

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REF-A115

THE EFFECTS OF THE TIMING OF INDUCTION ON THE METABOLISM AND PRODUCTIVITY OF CHO CELLS

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KEY WORDS:
CHO CELLS / ANTIBODY / INDUCIBLE EXPRESSION / 13C-METABOLIC FLUX ANALYSIS

BACKGROUND AND NOVELTY:
In the development of an efficient biphasic process, the timing of induction is one of the most crucial factors to optimize, as the product yield will ultimately be determined by both the cumulative viable cell density and the cell specific productivity. These two factors must be carefully balanced, as they depend and impact on the culture environment, most notably substrate and toxic by-product concentrations. In this work, we have investigated how the conditions at induction affect the cells' physiological state and the ensuing process productivity.

EXPERIMENTAL APPROACH:
We have employed a recombinant CHO cell line producing an antibody and harboring an efficient cumate-inducible expression system. Cells taken at different stages of growth were transferred and induced in fresh medium at their corresponding cell densities, and the kinetics of growth, nutrient consumption and product formation were compared during the production phase. Cultures induced respectively at low and high cell densities were further characterized by 13C-metabolic flux analysis, whereby cells were fed with mixtures of various 13C-labeled substrates and the mass isotopomer distributions of secreted amino acids and organic acids were determined by mass spectrometry.

RESULTS AND DISCUSSION:
Inductions carried out at low cell densities achieved lower maximum cell concentrations, but exhibited higher cell specific productivity and greater culture longevity, and ultimately led to increased final product titers. A number of key intracellular fluxes were found to be affected by the cell density at induction and the corresponding availability of nutrients. While glucose utilization was more efficient in high cell density cultures, with notably a greater fraction of pyruvate entering the TCA cycle, the catabolic rates of most amino acids were found to be reduced. Such analysis is needed to guide the rational development of biphasic processes, in particular to support the design of a balanced and efficient feeding protocol post-induction.

REF-A116

RAPID CHO CULTURE PROCESS DEVELOPMENT FOR PRE-CLINICAL AND CLINICAL MANUFACTURING USING A NOVEL FEED FORMULATION APPROACH

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KEY WORDS:
CHO / CELL CULTURE / FED-BATCH / MONOCLONAL ANTIBODY

BACKGROUND AND NOVELTY:
Robust fed-batch processes are required to sustain CHO cell-specific productivity late in culture when recombinant protein titers can plateau due to nutrient depletion. Maintaining high specific productivity and achieving scalable production targets rapidly can be challenging. Utilizing traditional optimization approaches often lead to large volume additions, feed solutions with extreme pH, and the use of undefined or otherwise problematic ingredients. To overcome these challenges, we have explored a novel technology for combining key nutrients at concentrations previously unattainable. This functional additive for CHO fed-batch processes boosts specific productivity by delivering key nutrients at high concentrations and neutral pH. With minimal process optimization, low volume additions have resulted in over twofold improvement in product titers for several CHO processes.

EXPERIMENTAL APPROACH:
In this study the functional additive was included during process development for two mAbs expressed in CHO. After proof-of-concept experiments in shake flasks and bench-top bioreactors, the process was then scaled up through pilot and manufacturing scales to generate multi-kilogram quantities of clinical trial material.

RESULTS AND DISCUSSION:
Use of the additive in shake flasks and bench-top bioreactors resulted in up to 70% titer enhancement compared to process without the additive. In all cultures, lactate consumption was observed after reaching peak cell density, allowing pH to be naturally maintained close to neutral. This permitted minimal or, on several occasions, zero alkali addition for pH control thereby resulting in manageable levels of osmolality and pCO2 at larger scales. Product titers observed up to about 4 g/L for the two products were conserved across scales. These results led to a rapid process development strategy allowing shortened timelines to reach the clinical trial phase for projects.

DEVELOPMENT OF A CHEMICALLY DEFINED FATTY ACID SUPPLEMENT FOR CHO MEDIA

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KEY WORDS:
CHO CELLS / FATTY ACID / CHEMICALLY DEFINED MEDIUM / BATCH FERMENTATION / FED BATCH FERMENTATION

BACKGROUND AND NOVELTY:
The use of culture media free of animal derived components for production of biopharmaceuticals has become state of the art. Newer development led to the usage of chemically defined media. This absence of any peptones may decrease the cell performance, resulting in lower cell density, viability and titer. For the development of NBEs and especially for biosimilars the cost of goods are a main driver for the selection of a production process. On the other side quality attributes of a product need to be defined and kept over the course of development and following market production.
Lots of work was done to increase productivity, with a strong focus on nutrients and vitamins. One main other performance driver are fatty acids. While nutrients can be easily solubilized, fatty acids need a carrier in order to be available for the cells. Both, the carrier as well as the fatty acids, need to be available in the necessary quality; they should be non-toxic, non-costly, and highly effective. In total 9 fatty acids and one carrier were identified for the development of the presented supplement.

EXPERIMENTAL APPROACH:
After identifying the optimal carrier, the different fatty acids were tested in terms of performance increase in several commercial available and in-house media. In a second step the best candidates were further optimized in DOE based experiments using several CHO production cell lines. The chosen conditions were batch and fed-batch mode in shake flasks as well as bioreactor set-ups.

RESULTS AND DISCUSSION:
Using the chemically defined fatty acid mixture supplement in various concentrations, the productivity of CHO cells increased more than 50 percent in batch and fed-batch mode. The achieved quality of the produced products remained within the quality target profiles.

The designed fatty acid mixture supplement decreased the cost of goods by 10 to 50% for the different processes, giving an economical advantage and increased likelyhood for the success of a given product.

EFFECTS OF PERFUSION PROCESSES UNDER LIMITING CONDITIONS ON DIFFERENT CHINESE HAMSTER OVARY (CHO) CELLS

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KEY WORDS:
CHINESE HAMSTER OVARY (CHO) CELLS / CULTIVATION / PERFUSION / GLUCOSE LIMITATION

BACKGROUND AND NOVELTY:
The use of perfusion culture to generate biopharmaceuticals is an attractive alternative to fed-batch bioreactor operation. The process allows for generation of high cell densities, stable culture conditions and a short residence time of active ingredients and has facilitated the production of sensitive therapeutic proteins. However, challenges remain for efficient perfusion based production at industrial scale, primarily the complexity of the required equipment and the strategies adopted for downstream processing. There is also a need to increase productivity during perfusion-based expression. We have shown previously that one effective way to enhance the cell specific productivity is via glucose limitation. However, the mechanisms leading to an increased productivity under these glucose limiting conditions, are still unclear. Preliminary studies using proteomic analysis indicated changes in the histone acetylation.

EXPERIMENTAL APPROACH:
In order to further explore these observations we have used a perfusion based cultivation of a recombinant CHO cell line producing Mucin 2 under pO2- and pH-controlled conditions. For cell retention a 20 µm internal spin filter was used.

RESULTS AND DISCUSSION:
Using this process we have reached viable cell densities of 1.4 · 10^7 cells /mL in a 24 day perfusion run. In the course of this cultivation, the cells underwent a metabolic shift towards more efficient glucose metabolism, resulting in drastically reduced lactate formation. Interestingly cell specific productivity increased by 90 % during this time.
To further demonstrate the applicability of this approach we are also conducting perfusion cultures using a range of unrelated CHO subclones. To analyse the impact of limiting conditions on the transcriptome of CHO cells, a microarray will be used. This proprietary CHO microarray covers more than 41,304 different probes to elucidate the reasons for the increase in cell specific productivity.

REF-A119

IMPROVEMENT OF PRODUCTION RATE ON RECOMBINANT CHO CELLS IN TWO-STAGE CULTURE

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KEY WORDS:
CHO / CR1 / CELL CYCLE / LOW TEMPERATURE CULTURE / FACS

BACKGROUND AND NOVELTY:
Cultivation temperature is one of the most important parameters that influence cell growth and recombinant protein production. Recombinant CHO (rCHO) cells are usually cultivated at 37 °C. Although low temperature cultivation below 37 °C decrease specific growth rate, it is known there are many beneficial effects in low temperature cultivation, such as high viability for a longer period, reducing the consumption rate of glucose and glutamine, and reducing the oxygen consumption rate. Unlike the specific growth rate, effects of low temperature cultivation on specific productivity rate are not so clear. We studied low temperature cultivation effects on sCR1 productivity in rCHO cells and report here. CR1, a human complement receptor type1 (C3b/C4b receptor), is a poly morphic membrane glycoprotein and sCR1, a soluble form of CR1.

EXPERIMENTAL APPROACH:
A 400 mL batch cultivation of rCHO cells in non-serum medium was carried out in a temperature range between 37 and 30 °C. Flow cytometric analysis was applied in order to analyze the cell cycle distribution.

RESULTS AND DISCUSSION:
Compared to 37 °C-cultivation, lower specific growth rate, maintaining higher viability, lower glucose and glutamine utilization, and lower lactate and ammonia production were observed in the lower temperature cultivations. On the contrary it was shown that the sCR1 production rate in 37 °C-cultivation was lower than that in lower temperature cultivation. Cell cycle analysis revealed that the ratio of G0/G1 phase and the specific production rate of sCR1 increase in lower temperature culture. We proposed a two-stage culture that cultivation was carried out at 37 °C and then a culture temperature become lower. We report that the final sCR1 concentration by a two-stage culture (37 to 33 °C) was double compared as a constant temperature culture at 37 °C.

REF-A120

RICE BRAN EXTRACT (RBE) AS SUPPLEMENT FOR CELL CULTURE

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KEY WORDS:
SUPPLEMENT / SERUM-FREE CULTURE / MAMMAL-FREE CULTURE / RICE BRAN / CHO

BACKGROUND AND NOVELTY:
In mammalian cell culture, fetal bovine serum (FBS) or proteins obtained from mammals are usually supplemented to culture media. Since the use of animal-derived components may cause an infection of virus and other pathogens, alternative supplement derived from non-mammals is eagerly required in cell culture for producing biotherapeutics and for cell therapy. As an alternative supplement, we focused on rice bran extract (RBE), because rice bran, by-product of milling in the production of refined white rice, contains abundant nutrients and proteins.

EXPERIMENTAL APPROACH:
RBE was extracted from rice bran in an alkaline solution, precipitated with acid, and subsequently freeze-dried. To test the effect, RBE was supplemented to the culture of hybridoma cells, Chinese hamster ovary cells (CHO-DP12), hepatoma HepG2 and HeLa. The cells were cultured in 24 well plate (Sumitomo Bakelite, Japan) with 1 ml ASF104 medium (Ajinomoto, Japan) containing RBE or BSA (Wako, Japan) as positive control. In order to identify the growth factor(s) in RBE, fractionations were performed using UF membranes.

RESULTS AND DISCUSSION:
On growth and monoclonal antibody production of hybridoma cells, RBE had desired effect and the effect of RBE was superior to that of BSA. Similarly, to CHO-DP12 cells, addition of RBE exhibited increased cell growth and improved the productivity of humanized antibody. Growth of HepG2 and HeLa cells were also enhanced in the presence of RBE.
Fractionated RBEs by UF membranes were also tested. The fraction of RBE more than 30 kDa improved the proliferation of hybridoma cells and the level was superior to that of whole RBE, while the fraction less than 30 kDa inhibited the proliferation. This results suggest that in RBE, some lower molecular inhibitor(s) and higher molecular growth factor(s) would be contained.
We provide the first evidence that RBE is an attractive culture supplement to improve the proliferation and the production of mammalian cells.

REF-A121

CHEMICAL CHAPERON SUPPRESSES THE ANTIBODY AGGREGATION IN CHO CELL CULTURE

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KEY WORDS:
CHINESE HAMSTER OVARY (CHO) CELL / ANTIBODY PRODUCTION / BISPECIFIC DIABODY / ANTIBODY AGGREGATION / CHEMICAL CHAPERON

BACKGROUND AND NOVELTY:
Quality control of therapeutic antibodies is one of the most important topics in the manufacturing process [1]. Aggregation of antibodies could be generated at different steps of the manufacturing process, posing the fundamental problem for quality control [2]. An addition of chemical chaperons is known to suppress the protein aggregation, and trehalosa, non-reducing sugar formed from two glucose units with -1,1 linkage, is effective co-solute for anti-aggregation. In this study, we investigated the anti-aggregation effect of trehalose during the cultivation process of Chinese hamster ovary (CHO) cell line producing humanized IgG-like bispecific antibody. The bispecific diabody is the promising candidate for next-generation therapeutic antibody because of dual functionality, whereas it shows aggregation tendency.

EXPERIMENTAL APPROACH:
Serum free-adapted CHO Top-H cell line [3] was cultivated in suspension. Produced diabody was purified by protein A chromatography. Biophysical properties were assessed by CD spectroscopy and size exclusion chromatography (SEC). Antibody concentration and the transcript level were assessed by ELISA and quantitative real-time PCR, respectively.

RESULTS AND DISCUSSION:
We successfully constructed the 150 mM trehalose-adapted CHO cells. Cell growth was strongly affected by trehalose; the specific growth rate and maximum cell density was decreased. On the other hands, the antibody concentration and the relative antibody mRNA level were enhanced. Trehalose may inhibit cell growth and enhance productivity in antibody-producing CHO cell. SEC analysis showed that the formation of large aggregates (LA) was significantly suppressed by trehalose addition. CD measurement revealed that the secondary structure of LA was misfiled structure with non-native -strand, whereas the structure of dimer was monomer-like structure. It was suggested that trehalose would suppress the the transition form dimer to LA in the cell culture process.

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REF-A122

DYNAMICAL ANALYSIS OF ANTIBODY AGGREGATION IN THE CHO CELL CULTURE WITH THERMO RESPONSIVE PROTEIN A (TRPA) COLUMN

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KEY WORDS:
CHINESE HAMSTER OVARY (CHO) CELL / BISPECIFIC DIABODY / ANTIBODY AGGREGATION / THERMO RESPONSIVE PROTEIN A (TRPA)

BACKGROUND AND NOVELTY:
Aggregation of therapeutic antibody is a common issue encountered in the manufacturing process [1]. Exposure to low pH condition during protein A affinity chromatography induces the antibody aggregation, raising the necessity of novel strategy to reduce the antibody aggregation in affinity purification process. In the presentation, we propose the novel affinity purification system, Thermo Responsive Protein A (TRPA) column. TRPA column make it possible to elute the antibody without low pH condition, because the engineered protein A ligand unfolds at moderate temperature. We evaluate the performance of the TRPA column in the purification of humanized single-chained IgG-like bispecific diabody-Fc (scDb-Fc) from the culture media of Chinese hamster ovary (CHO) cell line. Furthermore, we applied the TRPA column to the aggregation analysis of scDb-Fc during the CHO cell culture process.

EXPERIMENTAL APPROACH:
Serum free-adapted CHO Top-H cell line producing the scDb-Fc [2] was cultivated in suspension. Produced diabody was purified with thermo responsive protein A (TRPA) column. Aggregation of scDb-Fc in the cell culture process was analyzed by size-exclusion chromatography (SEC) and CD spectroscopy.

RESULTS AND DISCUSSION:
Compared to the conventional protein A, TRPA purification showed no precipitation of aggregated scDb-Fc. The complex multi-peak including the antibody fragmentation was observed in SEC analysis of TRPA-purified antibody, correctly reflecting the status of antibody aggregation during the cell culture process. Based on the property, we observed the time course of the aggregates formation of the scDb-Fc in the cell culture process. The amount of aggregated scDb-Fc including the high-order aggregates and the dimer was increased with the cultivation time and the concentration of scDb-Fc in the media. TRPA column is applicable to the aggregation analysis, and would make it possible to elucidate the aggregation mechanism during the cell culture process.

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REF-A123

OPTIMIZED PLATFORM MEDIUM AND FEED FOR RECOMBINANT rCHO CELL LINES USING THE CHEF 1® EXPRESSION SYSTEM

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KEY WORDS:
CHO / CHEF1 / CHEMICALLY-DEFINED / MONOCLONAL ANTIBODY / CELL CULTURE PLATFORM MEDIA

BACKGROUND AND NOVELTY:
Chinese Hampster Ovary (CHO) cells are widely used in biomanufacturing and biomedical research to produce proteins of clinical significance. The enviorment the cells grow in to produce these proteins is complex and varies across the industry. One key variable in production processes is the cell culture medium used. Media can include chemically-defined components such as amino acids, vitamins, lipids, metal salts, and buffers. In addition, undefined components such as proteins, serum, or hydrolysates may be added. To reduce complexity and increase consistency, chemically-defined formulations are preferred and can be developed and optimized for a given cell line. While a medium and feed can be optimized for every cell line/clone, developing a platform system provides a cost-effective option while ensuring a high level of growth and productivity.

EXPERIMENTAL APPROACH:
In this collaboration, a single animal origin-free, chemically-defined base platform medium and three synergistic feed media were developed for use with recombinant CHO cell lines engineered using the CHEF1® expression system to produce monoclonal antibodies.

RESULTS AND DISCUSSION:
Two cell lines were tested yielding an average of about a 1.6 fold improvement in titer over the undefined media, allowing a platform process to be developed and integrated into the culture strategy.

REF-A124

CHARACTERIZATION OF MOBUIS® SENSORREADY TECHNOLOGY: THE EFFECT OF A FLOW-THROUGH EXTERNAL SENSING CIRCUIT ON CELL GROWTH AND METABOLISM

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KEY WORDS:
SHEAR STRESS / CELL VIABILITY / PRODUCTIVITY

BACKGROUND AND NOVELTY:
To address the need to integrate new sensor technologies into large-scale cell processes, a novel bioreactor monitoring approach has been developed. The Mobius® SensorReady technology is an external circuit containing sampling ports and a configurable number of probe ports that are connected to the Mobius® CellReady bioreactors at the time of use. This modular design provides the user with ultimate flexibility to monitor and control their bioreactor processes with a variety of both conventional and cutting edge sensor technologies. A fixed flow rate of 3 LPM is consistently maintained in the loop using a low shear, bearingless centrifugal rotary pump which has been demonstrated previously to provide accurate monitoring of the bioreactor.

EXPERIMENTAL APPROACH:
Along with the integration of this technology into routine cell culture processes, come concerns about the design's impact on cell culture performance that could result from additional shear stress imparted on the cells. These concerns were addressed using small-scale 3 L bioreactors configured with an external loop containing the low shear pump. The studies conducted examined the impact of the pump on growth, metabolism and productivity of an IgG producing CHO cell line. Using these small-scale test beds, the effect of increasing pump speed (i.e., higher flow rates) on overall cell health was evaluated. Finally, results from larger scale bioreactors compared the different recycle rates of the pumps at the different scales.

RESULTS AND DISCUSSION:
The data generated from these studies demonstrate that the low shear pump has a negligible impact on overall cell health and productivity when used as designed. Evaluation of the system at flow rates outside of the operating window impacts cell growth in a dose-dependent manner. The work presented herein show that the optimized 3 LPM flow rate provided with the platform, offers a significant safety factor from the damaging effects of shear stress and further support the implementation of this approach.

IDENTIFICATION OF MITOGENIC FACTOR IN RICE BRAN FOR BETTER MAMMALIAN CELL CULTURE

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KEY WORDS:
RICE BRAN / MITOGEN / SERUM-FREE / HYDROLYSATE / PROTEIN

BACKGROUND AND NOVELTY:
In cell culture for biopharmaceutical production, serum-free culture is required in order to avoid the risks associated with components of mammal origin such as BSE. Although many serum-free medium have been developed, there is yet room for improvement and currently, protein hydrolysates from crops are widely used as additives to improve the culture. We found that rice bran extract (RBE), not hydrolysate, successfully improved the proliferation of various cells as well as recombinant protein production of CHO cells when RBE was added into serum-free culture. RBE contains various components such as proteins and the factors activating mammalian cells are not identified yet. In this study, we aim to identify the effective factor in RBE.

EXPERIMENTAL APPROACH:
The major components of RBE are proteins (50%), glucides (20%), and lipids (5%). Because proteins are most abundant component and because the SDS electrophoresis study implied that much of proteins would not be digested, we expected that some of the proteins in RBE would be the effective factor or the mitogen. In order to test this hypothesis, RBE was autoclaved or digested with trypsin, and then added to the culture of hybridoma cells to assay their mitogenic activity. In detail, RBE was autoclaved at 121ºC for 20 minutes, or digested with 0.115 mg/mL trypsin at 37 ºC for 24 hours. The treated RBE was supplemented to the culture of a murine hybridoma cell line 2E3-0 in serum-free ASF104 medium. After cultured for three days, viable cell number was determined by trypan blue dye exclusion with hemocytometer.

RESULTS AND DISCUSSION:
The cells treated with autoclaved RBE had the lower growth rate than the cells with unheated RBE did, suggesting that heat-sensitive ingredients would be effective factors. Similarly, the cells treated with trypsinized RBE had the lower growth rate than the cells treated with undigested RBE did. These results suggest that effective factors in RBE would be some proteins.

GENERATION OF THE BI-HEX®-GLYMAXX® CELL LINE FOR EFFICIENT PRODUCTION OF NEXT GENERATION BIOMOLECULES WITH ENHANCED ADCC ACTIVITY

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KEY WORDS:
ADCC-ENHANCED BIOTHERAPEUTIC / FUCOSYLATION / GLYCOPROFILE ENGINEERING / BIOBETTER MANUFACTURING

BACKGROUND AND NOVELTY:
Despite the succes story of therapeutic monoclonal antibodies (mAbs), a medical need remains to improve their efficacy, for example antibody dependent cellular cytotoxicity (ADCC). A mAb´s ADCC activity can be enhanced either by mutating amino acids in its Fc region or by modifying its Asn297 N-linked glycan. Unlike mutagenesis, glycoengineering is unlikely to increase immunogenicity of the drug. One way to enhance ADCC is to reduce the level of glycan fucosylation. Therefore, defucosylated antibodies are of major interest for the biopharmaceutical industry.

EXPERIMENTAL APPROACH:
Boehringer-Ingelheim has pursued the glycoengineering approach by engineering our platform BI-HEX® CHO host cell to generate the novel BI-HEX®-GlymaxX® host cell. This was achieved in collaboration with ProBioGen, Germany, taking advantage of their GlymaxX®-technology which is based on the bacterial enzyme RMD which, when expressed in BI-HEX®, turns a fucose biosynthesis intermediate into rhamnose, thereby inhibiting de-novo fucose biosynthesis.

RESULTS AND DISCUSSION:
The BI-HEX® host cell line was stably transfected with the RMD enzyme and stable clones expressing RMD were selected. The clones were analysed for stability of RMD expression over time, glycoprofile structure and ADCC activity of mAbs produced by these clones. The data showed a very significant reduction of fucose levels in the glycan structure which correlated nicely with enhanced ADCC activity. Depletion studies of RMD were also done to show that this could be efficiently depleted during downstream purification of the mAb. Furthermore, we examined the growth and cultivation properties of the modified BI-HEX®-GlymaxX® cells to ensure that the engineered host cell maintained the favourable manufacturability properties of BI-HEX®. In summary, the obtained data indicate that BI-HEX®-GlymaxX® is a host cell of choice for generation of ADCC-enhanced therapeutic mAbs.

REF-A127

USING RICE BRAN EXTRACT (RBE) AS SUPPLEMENT FOR MESCENCHYMAL STEM CELLS (MSCS) IN SERUM-FREE CULTURE

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KEY WORDS:
MESCENCHYMAL STEM CELLS / RICE BRAN / SERUM-FREE / OSTEOBLASTIC DIFFERENTIATION / EXPANSION

BACKGROUND AND NOVELTY:
Currently, therapies using multipotent mescenchymal stem cells (MSCs) are tested clinically for various disorders, including cardiac disease. However, conventional culture media contain fetal bovine serum (FBS) and so the concerns about amphixenosis remain. Therefore, developing animal derived factor-free media are desired. We found that rice bran extract (RBE) improved the proliferation of various cells and the cellular functions. In this study, we tested the effect of RBE on MSCs in serum-free culture.

EXPERIMENTAL APPROACH:
MSCs obtained from the bone marrow of Wistar rats were cultured under conventional -MEM with 15% FBS or serum-free STK1 medium, supplemented with or without RBE for 3 days at passage from 1 to 3. Subsequently, some of the cells were lysed to be analyzed the maintaining MSC markers with real-time PCR, and the others were cultured in the osteoblastic differentiation medium for 28 days. To quantitate the differentiation ability, the cells were stained with Alizarin Red S and analyzed by using Image J.

RESULTS AND DISCUSSION:
When cultured in serum-free STK1 medium supplemented with RBE, MSCs aggregated, but did not without RBE, implying that RBE would affect cell-cell interaction. According to analysis by real-time PCR, the expression of MSC markers CD 44, 105 and 166 were maintained in the cells cultured in STK1 as well as in conventional FBS culture. Although the MSC markers were expressed, MSCs cultured in STK1 failed to differentiate. Addition of RBE into the STK1 medium improved this decline of differentiation; in the presence of RBE, MSCs cultured in serum-free medium osteoblastically differentiated.

REF-A128

NOVEL PERFUSION SINGLE-USE-BIOREACTOR WITH INTEGRATED CELL SEPARATION DEVICE: CELLTANK™

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KEY WORDS:
PERFUSION / SINGLE-USE-BIOREACTOR / ON-LINE BIOMASS SENSOR / CHO CELLS / ANTIBODY EXPRESSION

BACKGROUND AND NOVELTY:
A new perfusion integrated Single-Use-Bioreactor (SUB) culture system CellTank™ has been developed for highly efficient production of recombinant products from mammalian cell cultures. In this system, both adherent and suspension cells are harboured in a novel matrix, CellCore, composed of two parallel stacked matrix discs caged each in envelopes and immersed in a reservoir. CellTank™ can be scaled up by enlarging the matrix disc diameter and increasing the number of matrix discs, generating a working volume spanning >1:1000 from few milliliters to 15L.

EXPERIMENTAL APPROACH:
In this study, suspension-adapted recombinant Chinese Hamster Ovary (CHO) cells producing IgG monoclonal antibody were cultivated in the CellTank 2202 prototype, SUB with 150cm3 CellCore matrix. A real-time biomass sensor from FOGALE nanotech using the dielectric properties of living cells was used to measure the live cell density. The operation and performance of this setting were studied in perfusion cultivations.

RESULTS AND DISCUSSION:
A high viable cell density measured as 2E8 pF/cm was achieved at perfusion rate 10 RV/day (Reactor Volume per day) during the first run, where 1 pF/cm is equivalent to 1E6 viable cells/mL. In the second run, viable cell density of 1.3E8 pF/cm was maintained for 11 days at perfusion rate 8 RV/day-10 RV/day at low temperature. The cell specific productivity was comparable or higher than the one measured in batch culture in Erlenmeyer flasks. The volumetric IgG productivity was up to 61 times higher than in Erlenmeyer flasks batch cultures. There was no retention of IgG in the CellCore matrix. As a bench-top Single-Use-Bioreactor, the CellTank™ system integrating the cell separation device can work long-term and stably at cell density above 1E8 viable cells/mL without IgG retention in the matrix. Being easy and handy to operate, CellTank™ offers a solution alleviating technical and sterility challenges occurring in traditional perfusion processes and makes an excellent choice for biotherapeutics production.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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IMPROVED FED BATCH BIOPROCESSES WITH CONCENTRATED FEEDS FOR THE LONG TERM CULTIVATION OF CHO CELLS

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KEY WORDS:
CONCENTRATED FEED / FED BATCH PROCESS / MODIFIED AMINO ACIDS

BACKGROUND AND NOVELTY:
Fed batch culture bioprocesses have emerged as the predominant mode for the production of recombinant proteins. In these cultures concentrated feeds are added during cultivation to prevent nutrient depletion, extending the growth phase and increasing cell and product concentrations. One limitation in these bioprocesses arises from the low solubility or stability of some compounds at high concentration, in particular amino acids. The work presented here describes new strategies to overcome solubility and stability issues of common amino acids like tyrosine or cysteine.

EXPERIMENTAL APPROACH:
Critical amino acids for fed batch processes were subjected to either physico-chemical or chemical modifications to enhance their solubility / stability. After complete physicochemical characterization, the impact of these new molecules on cell growth, cellular uptake as well as quality of the final molecule was evaluated.

RESULTS AND DISCUSSION:
Modified amino acids demonstrated a better solubility and stability in neutral media and concentrated feed in comparison to control molecules. The cellular uptake by CHO cells was highly dependent on the chemical modification whereas cell growth and titer obtained with lead molecules were comparable or even better as controls. Finally, this work demonstrates that modified amino acid can be used successfully in highly concentrated feeds to improve next generation fed batch processes.

ACCELERATING STABLE RECOMBINANT CELL LINE DEVELOPMENT USING TARGETED INTEGRATION

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KEY WORDS:
TARGETED INTEGRATION / RECOMBINASE MEDIATED CASSETTE EXCHANGE / CELL LINE DEVELOPMENT / CANDIDATE SCREENING / RMCE

BACKGROUND AND NOVELTY:
Targeted integration (TI) allows fast and reproducible genetic modification of well characterized previously tagged host cells thus generating producer cells with predictable qualities. In contrast to commonly low productivities of cell lines generated by TI, we developed a system for CHO cells leading to productivities of >1 g/L within weeks using the TurboCell™ platform.

EXPERIMENTAL APPROACH:
CHO K1 cells were tagged with a GFP expression construct flanked by recombinase target sites. Following GFP based FACS enrichment and cloning of the producer cells, more than 4000 clones were screened for growth, productivity, GFP expression stability and integration status of the GFP expression cassette. The most promising clones were chosen for recombinase mediated cassette exchange (RMCE) of the GFP gene by the genes of several model proteins including IgG1. Homogenous rProt (recombinant Protein) expressing pools were generated by FACS and used for early rProt production. Clones from those pools were cultivated in shake flasks and bioreactors to analyze their growth, productivity and rProt quality. The integrated gene copy number was screened as well as the occurrence of unintended random integration.

RESULTS AND DISCUSSION:
Within 3 weeks from transfection, rProt producer cells were FACS sorted to purities of >95 %. These cells showing successful TI were suited for high quality rProt material production in fed batch runs at the g/L scale. Clones generated thereof behaved similar to the pools in terms of rProt productivity and quality, cell growth and metabolism. From analyzed clones a mean of 85 % showed successful RMCE with no unintended random integration. Cellular properties and productivities of the clones were as expected and variations in between the clones were marginal. Thus the TurboCell™ system reduces clone screening efforts to a minimum allowing the simultaneous production of multiple rProts in stable CHO cells with optimal use of resources.

REF-A131

IMPROVED RECOMBINANT PROTEIN PRODUCTION USING SMALL MOLECULE ENHANCERS AND ANTI-OXIDANTS IN RECOMBINANT CHO CELLS

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KEY WORDS:
BIOPROCESS OPTIMIZATION / BIOPHARMACEUTICAL / SMALL MOLECULE ENHANCERS / ANTIOXIDANT

BACKGROUND AND NOVELTY:
Increasing the volumetric titer of recombinant therapeutic proteins is a key objective in bioprocess manufacturing development. Carboxylic acids have demonstrated the ability to improve synthesis of recombinant proteins in industrially important cell lines such as Chinese Hamster Ovary (CHO) cells. The improvement in the production of recombinant proteins has been linked in a number of molecules to the inhibition of histone deactylase, leading to increased transcription of genes. However, carboxylic acids have been shown to promote an apoptotic response in CHO cell culture. Pentanoic acid is a carboxylic acid that has demonstrated lower apoptotic response in culture relative to the carboxylic acid butyrate, while maintaining the ability to improve recombinant protein production. Supplementation of cultures with anti-oxidants has shown the ability to reduce the apoptotic response to carboxylic acid supplementation leading to increased therapeutic protein production. Supplementation of cultures with pentanoic acid and select antioxidants has the potential to increase protein production above that seen with pentanoic acid alone.

EXPERIMENTAL APPROACH:
Pentanoic acid and butryic acid supplemented cultures of a CHO cell line, producing a recombinant IgG, were studied with supplementation of the antioxidant n-acetyl cysteine. Studies were performed in time-course experiments looking at growth, productivity, and apoptosis.

RESULTS AND DISCUSSION:
Pentanoic acid was shown to reduce the number of cell entering early apoptosis relative to butyric acid by 15.4%. The supplementation of butyric acid and pentanoic acid treated cultures with n-acetyl cysteine reduced the population of cells entering early apoptosis by 5.3% and 10.0%, respectively. A 19.5% increase in productivity was observed in pentanoic acid treated cultures when supplemented with n-acetyl cysteine. This research provides evidence for a culture supplementation method that can be used in the optimization of biopharmaceutical manufacturing processes.

REF-A132

STATE OF THE ART MEDIA DEVELOPMENT AND IMPLEMENTATION TO ENABLE HIGHER TITERS IN MAMMALIAN BIOPHARMACEUTICAL PROCESSES

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KEY WORDS:
RATIONAL MEDIA DESIGN / CELL CULTURE PROCESS / HIGH TITER / HIGH-THROUGHPUT TECHNOLOGIES / DISPOSABLE BIOREACTORS

BACKGROUND AND NOVELTY:
Medium plays a major role in developing high performance mammalian cell culture processes. Rational media design was used to develop a novel animal component free and chemically defined medium for superior mammalian cell culture performance and final product titer.

EXPERIMENTAL APPROACH:
By applying advanced systems biotechnology tools such as metabolomics, gene expression analysis (DNA chip technology and next generation sequencing), and stochiometric media design we have developed a balanced chemically defined medium. This rational approach was supported by the implementation of high-throughput screening technologies like the ambr system and by advanced “at-line” analytical tools.

RESULTS AND DISCUSSION:
Superior cell growth of various cell lines with peak cell densities up to 45 million cells/mL and final titers up to 8g/L in fed-batch cultures were achieved by implementing the new media platform in several projects. These high cell density processes were successfully harvested for further processing. Further, we will present data from the successful scale-up of processes to commercial scale for efficient production of biopharmaceuticals in stainless steel and disposable bioreactors.

PROFILING OF GLYCOSYLATION GENE EXPRESSION IN CHO FED-BATCH CULTURES IN RESPONSE TO GLYCOSYLATION ENHANCING MEDIA COMPONENTS

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KEY WORDS:
CHO / GLYCOSYLATION / TRANSCRIPTOME

BACKGROUND AND NOVELTY:
Chinese Hamster Ovary (CHO) cells are the predominant cell substrate used in the biopharmaceutical industry for the production of therapeutic proteins. The glycosylation of therapeutic proteins in CHO cells is a critical parameter, one that needs to be extensively examined to ensure a consistent, effective and high-quality protein product. Currently, there are limited data exploring changes in glycosylation gene expression in response to cell culture media and feed conditions. We are using Next Generation RNA-Sequencing (RNA-Seq) technology to characterize and understand how gene expression can be affected by cell culture media components.

EXPERIMENTAL APPROACH:
In a previous study conducted with CHO-S and DG44 cells in shake flasks, addition of proprietary media components A and B resulted in a significant increase in G1F and G2F glycoforms when compared to controls while still maintaining comparable protein production. In the present study, we compared the changes in protein glycosylation to changes in glycosylation-related gene expression in response to media components. CHO-S and DG44 cells were grown in DASGIP (0.5L) bioreactors with and without components A and B. Cell growth, viability and IgG productivity were measured as a time course. IgG samples were analyzed for glycosylation status on an ABI 3500 and RNA samples were sequenced using RNA-Seq methodology on Ion Torrent PGM™. Whole transcriptome analysis of the samples were compared to targeted gene expression analysis focused on glycosylation-specific genes.

RESULTS AND DISCUSSION:
From these data, we estimated the differential gene expression profiles between CHO-S and DG44, changes in gene expression over time in culture, and functional differences associated with changes in culture media components. These findings will shed light into genetic mechanisms in CHO cells that could potentially be optimized through culture media and feed approaches to improve product therapeutic efficacy and half-life.

THE ROLE OF ADENOVIRUS REPLICATION STEPS ON THE PRODUCTION OF HELPER-DEPENDENT VECTORS

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KEY WORDS:
CANINE ADENOVIRUS TYPE 2 / HELPER-DEPENDENT VECTORS / GENE THERAPY / ADENOVIRUS PRODUCTION / VIRUS REPLICATION CYCLE

BACKGROUND AND NOVELTY:
Adenoviral Helper-dependent vectors (HDVs), devoid of any viral coding gene and thus eliminating the problems associated with residual viral gene expression, are promising tools for gene delivery applications, holding high transduction levels, gene expression for long periods of time and transfer of large DNA fragments. Despite these advantages, their production yields are at least 10-fold lower than the corresponding E1-deleted (ΔE1) vectors, constraining the development of large-scale production of clinical grade HDVs and the use of these vectors in patients. Moreover, the mechanisms underlying the low production of HDVs have never been unraveled.

EXPERIMENTAL APPROACH:
In this work, we analyzed which steps of the adenovirus replication cycle are altered in HDVs particles generation and investigated the expression of corresponding viral components by comparison with ΔE1 vectors. Given their attractive features to address fundamental neurobiological questions and to develop potential treatment of neurodegenerative disorders, we selected Canine Adenovirus type 2 (CAV-2) vectors.

RESULTS AND DISCUSSION:
The entry of HD CAV-2 genomes into the cell nucleus at early time-points was not impaired and the number of replicated viral genomes were found to be similar to those obtained with control ΔE1 CAV-2. However, gene expression analysis revealed that HD CAV-2 genome was transcribed at low levels. Western blot analysis during production suggests that the majority of viral proteins was expressed similarly in HD and ΔE1 CAV-2. Also, the evaluation of free viral genomes and full virions indicated that neither assembly nor packaging are limiting the generation of HDVs; however, the corresponding infectivity was 5-times lower than in control vector. These results indicate maturation step as the major bottleneck, dictating the system's productivity. The present study identified the key viral targets to be improved through vector or cell-line engineering towards the development of a robust production process for HDVs.

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REF-A136

A NOVEL HIGH-THROUGHPUT MEDIA DESIGN APPROACH FOR HIGH PERFORMANCE MAMMALIAN FED-BATCH CULTURES

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KEY WORDS:
MEDIA DEVELOPMENT / HIGH THROUGHPUT / FED-BATCH

BACKGROUND AND NOVELTY:
An innovative high-throughput medium development method based on media blending was successfully used to improve the performance of CHO cells in fed-batch cultures. The experiment was performed using a new cultivation system based on shaking 96 deepwell plates, a system which is predictive of fed-batch performance in bioreactor cultures. Each individual well contains a micro-scale suspension culture which is operated according to the same protocol as the manufacturing fed-batch platform (i. e. same culture duration and feeding regime).

EXPERIMENTAL APPROACH:
Starting from a proprietary chemically defined medium, 16 new formulations were designed with the same components but different concentrations of each component. More precisely, 43 out of a total of 47 components were varied at 3 different levels in each of the 16 formulations. The new formulations were adjusted to the same pH and osmolality and subsequently mixed by DoE in a binary mode using a robotic liquid handling platform. All resulting 376 blends were then tested during both cell expansion and fed-batch production phases in one single 96-deepwell plate experiment using a model antibody expressing cell line. The output of the experiment was analyzed using different statistical methods. A simple ranking of conditions was first used as a quick approach to select new formulations with promising features. Then, a prediction of the best mixes was done with Design Expert with the aim of maximizing both growth and productivity. Finally, a multivariate analysis was used to identify individual potential key components for further optimization.

RESULTS AND DISCUSSION:
The potential for performance improvement of a small number of tested and predicted media formulations was then successfully confirmed at larger scales and with a panel of antibody producing cell lines. The 96-deepwell plate system and the high-throughput media design approach were conceived to ensure that the experimental output is compatible with the manufacturing fed-batch platform in use and to deliver new high performance media formulations in the shortest possible time frame.

REF-A137

UNDERSTANDING CELL CULTURE MEDIA IMPACT ON PROCESS ROBUSTNESS OF TARGETED GLYCAN MOIETIES

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BACKGROUND AND NOVELTY:
Over the past decade, notable advances in the characterization and control of post translational modifications (PTMs) to enhance specific therapeutic characteristics have continued to drive the development of many next-generation biopharmaceuticals. It has been widely reported that the quality of secreted therapeutic proteins is dependent on the consistency of attached glycan moieties. Our particular interest lies in understanding the potential role of energy metabolism in recombinant protein PTMs, specifically N-linked glycosylation. In this initial study, we establish the application of principal component analysis (PCA) in media development to generate and understand variability in targeted glycan moieties.

EXPERIMENTAL APPROACH:
The investigation was initiated by applying PCA to SAFC's library of chemically defined media. The analysis was employed to identify patterns in formulations, and express the data in such a way as to highlight media similarities and differences. Forty (40) divergent chemically defined SAFC formulations were selected for an initial media screen to establish diverse culture responses. SAFC's IgG-producing CHOZNTM-GS clones were selected as the mammalian bioproduction model. A high throughput analytical method using intact protein accurate mass data was employed to perform glyco-profiling. Data represents average relative glycoform distributions of purified protein sampled 6 days post-inoculation of batch cultures. Growth, IgG productivity, and metabolite production were studied using standard analyses.

RESULTS AND DISCUSSION:
Terminal galactosylation of the glycans varied as follows: 43-81% of the N-glycans have a G0 structure, 11-43% a G1 structure, and 1-12% a G2 structure. The results from this study validate the effectiveness of PCA in media development to ensure variable culture performance. This approach represents an important step towards understanding the impact of individual biochemical components on process robustness and enhancement of targeted glycan moieties.

REF-A138

DISPOSABLE ORBITALLY SHAKEN TUBESPIN® BIOREACTOR 600 FOR MAMMALIAN CELL CULTIVATION

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KEY WORDS:
DISPOSABLE BIOREACTOR / CHO CELLS / MIXING / MASS TRANSFER / POWER CONSUMPTION

BACKGROUND AND NOVELTY:
Orbitally shaken bioreactors are known to support the efficient cultivation of mammalian cells in suspension. For medium-scale cultures, a disposable tube with a ventilated cap and a nominal volume of 600 mL (TubeSpin® bioreactor 600) has recently been developed. This vessel has a conical bottom so that it fits into a standard swinging bucket rotor to facilitate cell harvest by centrifugation.

EXPERIMENTAL APPROACH:
In order to define the working conditions for the TubeSpin® bioreactor 600, we measured several engineering principles such as the volumetric power consumption, mixing time, mixing homogeneity, and gas transfer rate. In addition, we measured cell growth, recombinant protein production, and environmental culture conditions (pH, pO2, pCO2) over a range of shaking frequencies and working volumes using a CHO-derived cell line stably expressing a recombinant IgG antibody.

RESULTS AND DISCUSSION:
This bioreactor has mass transfer coefficient (kLa) values above 30 hr-1, mixing times of less than 20 s, and specific power consumption values up to 0.5 kW/m3 at shaking frequencies suitable for mammalian cell cultivation. Comparable cell growth was observed in a TubeSpin® bioreactor 600, a 1-L cylindrical glass bottle, and a 1-L Erlenmeyer shake flask at a working volume of 300 mL. Dissolved oxygen levels remained high in the TubeSpin® bioreactor 600, but declined in the other two vessels. This study shows the feasibility of the TubeSpin® bioreactor 600 for cell culture applications with suspension-adapted mammalian cells at volumes of 100 to 500 mL.

REF-A139

LARGE SCALE MANUFACTURING OF AAV VIRUS-BASED GENE THERAPY VECTORS: DEVELOPMENT OF A SCALABLE PROCESS USING SARTORIUS STEDIM BIOTECH TECHNOLOGY

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KEY WORDS:
AAV VECTORS / LARGE SCALE / STR / SINGLE USE TECHNOLOGY

BACKGROUND AND NOVELTY:
Adeno-associated virus (AAV) vectors were developed over the past decade as particularly promising gene transfer vectors for in vivo applications. They are vectors of choice for the development of gene therapy treatments for many rare diseases affecting various tissues including retina, central nervous system, liver, and muscle. This vector stays essentially episomal after gene transfer, making it safer due to the absence of insertional mutagenesis. The non-pathogenicity of AAV is a further advantage. Due to the sole availability of transfection methods characterized by limited scalability, for long time, this vector could only be produced at a small scale for research purposes and phase 1 clinical trials. However, since the development of scalable processes based on suspension cell culture and baculovirus expression system (Bev/SF9), this bottleneck is resolved and, from a technical point of view, large quantities of AAV vectors can be produced, thus, opening the possibility of producing AAV vector quantities required for whole body treatments.

EXPERIMENTAL APPROACH:
At Généthon, the AAV based gene therapy approach became conceivable only after the development of an easily scalable production system in bioreactors using the Sf9 insect cell/baculovirus expression system. This production process has been developed from the lab scale with the conventional glass bioreactor system (Biostat BDCU from Sartorius) to an industrial scale in single use technology (200L Cultibag STR, Sartorius).

RESULTS AND DISCUSSION:
Today at Généthon, this manufacturing system allows the production of purified AAV vectors of up to 2 x 10^15 vector genomes (total vg), rendering the manufacturing of AAV feasible for clinical purposes. All of these achievements as well as further process intensifications are urgently needed for the production of clinical doses for the treatment of neuromuscular diseases for which estimated doses of up to 10^14 vg/kg body mass are required. This poster presents the principles and achievements of the Sf9/baculovirus system for the production of AAV developed at Généthon with the Sartorius Stedim Biotech technology, highlighting the scalability from a 2L glass vessel biorector up to a 200L single use bioreactor.

REF-A140

HEK293 CELL CULTURE MEDIA STUDY: INCREASING CELL DENSITY FOR DIFFERENT BIOPROCESS APPLICATION

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BACKGROUND AND NOVELTY:

The increasing demand for biopharmaceuticals produced in mammalian cells has lead industries to enhance bioprocess volumetric productivity through different strategies. Among them, media development is one of major interest [1]. The work presented provides simple and efficient media supplementation approaches of commercial HEK293 cell culture media for two bioprocess scenarios, when animal derived components can be used and when its use is not desirable, achieving in both cases outstanding results never reported before in batch cultures.

EXPERIMENTAL APPROACH:

Several commercially available culture media recommended for HEK293, were evaluated in terms of maximal growth rate (μmax), maximal viable cell concentration (Xvmax) supported and recombinant adenovirus production. The effect on these parameters of the addition of FBS or/and animal derived component free (ADCF) supplements and their concentration was also assessed.

RESULTS AND DISCUSSION:

FBS depletion was acceptable only in one of the three media evaluated as the others reached higher cell densities when FBS was added (up to 7- fold increment of Xvmax). Regarding the screening of ADCF supplements, only one out of three supplements significantly enhanced cell growth. Cell Boost 5supplement (CB5) enabled to reach higher cell densities in all media tested (from 2- fold up to 5- fold increment). HyQ SFMTransFx- 293 was selected for the study of the best concentration of each supplement (5% v/v for FBSand 10% v/v for CB5 solution). At these concentrations, Xvmax achieved were (7.14±0.56*106 cell/mL) and (12.63±1.76*106 cell/mL) respectively. Interestingly, CB5 enabled to extend μmax phase while FBS increased μmax value. The supplements combination of 5% FBS and 10%CB5 resulted in a Xvmax as high as 16.77±0.70 x106cell/mL in batch culture. Additionally FBS increased up to fivefold the adenovirus production, whereas CB5 supplementation did not affect significantly, and the addition of both supplements almost doubled the viral production in comparison to basal medium. It is proposed that an increment of osmolarity due to the addition of both supplements might explain the slight reduction on adenovirus production in comparison to the addition of FBS solely [2].

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

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We would like to thank Dr. Amine Kamen (BRI- NRC, Canada) for kindly providing the HEK 293 cell line.

REF-A141

PRELIMINARY STUDIES OF CELL CULTURE STRATEGIES FOR BIOPROCESS DEVELOPMENT BASED ON HEK293 CELLS

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BACKGROUND AND NOVELTY:

The use of human embryonic kidney cells (HEK293) for recombinant protein or virus production has Gained relevance Along the Last years. They are specially recommended for transient gene expression and adenovirus or adeno- associated virus generation [1,2]. In order to achieve high volumetric productivities cell cultures strategies for high cell density cultures must be developed [3]. In this work a preliminary study of culture strategies is presented.

EXPERIMENTAL APPROACH:

We have compared batch operation to media replacement, punctual additions of nutritional supplement and fortified batch cultures on lab scale. Additionally, main carbon source and metabolic by- products profiles of the different cultures strategies studied were analysed and compared.

RESULTS AND DISCUSSION:

Characterization of HEK293 cell culture in batch operation was initially performed. It was encountered that cell growth was extended for 168h reaching approximately 7*106 cell/mL of cell density. Nevertheless, μmax was only maintained for 96h. Nutrient limitation arose as the first hypothesis for this decrease on cell growth rate. Therefore, punctual additions of nutritional supplement for HEK293 were carried out. Despite the supplement concentration and number of additions, the maximal viable cell concentration reached was about 17*106 cell/mL. The results suggested that by- product accumulation different from lactate could be limiting cell growth. Thus, complete media replacement (up to three replacements) was studied. Although this strategy enabled to extend μmax phase, the maximal cell density reached was similar to nutrient addition strategy. Taking into account all the results, nutrient depletion, metabolic by- product toxicity or osmolarity were discarded as causes of cell growth limitation. Fortified batch cultures corroborated the results and provided a simpler strategy for the platform used. Future work to be performed will be focused on controlled and monitored systems in order to evaluate a potential oxygen supply limitation.

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We would like to thank Dr. Amine Kamen (BRI- NRC, Canada) for kindly providing the HEK 293 cell line.

CULTURE AND CRYOPRESERVATION OF HEPARG CELLS: COMBINING THREE-DIMENSIONAL CULTURE APPROACHES AND ALGINATE ENCAPSULATION FOR IMPROVED LIVER FUNCTIONALITY

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BACKGROUND AND NOVELTY:

The drug development process is widely hampered by the lack of human models that recapitulate liver functionality and efficiently predict toxicity of new chemical compounds. The bipotent liver progenitor cell line HepaRG can be differentiated into cholangiocyte and hepatocyte-like cells that express major functions of mature hepatocytes. However, the existing protocols for differentiation are time consuming and rely on the presence of DMSO, which can limit toxicity screening and conceal the metabolic and synthetic aspects of liver functionality.
Herein, we present a three-dimensional (3D) strategy for improvement of liver functionality based on the encapsulation of aggregates of HepaRG cells and culture in the absence of DMSO. Moreover, alginate encapsulation was evaluated as a strategy for the cryopreservation of differentiated HepaRG, enhancing its´ potential to be used as a cell product for toxicity applications.

EXPERIMENTAL APPROACH:

HepaRG aggregates were encapsulated with two concentrations of alginate to evaluate the effects of matrix stiffness on 3D behavior of HepaRG cells. After culture for 2 weeks in the absence of DMSO, cells were induced with prototypical inducers and CYP450 expression and activities were analyzed. Additional hepatic features were assessed such as the expression and presence of hepatic proteins and bile canaliculli functionality. For the cryopreservation, the slow-rate freezing method was optimized and viability and hepatic functionality were assessed after thawing.

RESULTS AND DISCUSSION:

The 3D strategy applied leads to an improvement of basal activities and inducibility of CYP450 as well as other hepatic features compared to 2D protocols. Importantly, differentiation is DMSO-free, presents a 50% reduction in process time and can be coupled with efficient cryopreservation. Therefore, the presented strategy results in a highly functional and ready-to-use hepatic cell product with applicability in both hepatotoxicology and bioartifical liver support.

OTHER INFORMATION

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BIOLOGICS EXPRESSION SYSTEM OPTIMIZATION AND BIOPROCESS DEVELOPMENT

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BACKGROUND AND NOVELTY:

The development of optimized protein expression systems and processes is a key step in the successful production of biologics for research and development purposes, and for large-scale product manufacturing. Optimizing expression systems requires various disciplines and laboratories to work in a division-of-labor environment, exchanging biomaterials and data between molecular biology, cell science, engineering, assays and analytics, and bioprocess development groups.

EXPERIMENTAL APPROACH:

We present a novel data management and analysis platform for vector development, cell line development, cell line engineering, protein expression optimization and bioprocess development that resulted from a multi-year collaboration with leading pharma R&D laboratories.

RESULTS AND DISCUSSION:

The system enables a comprehensive tracking and annotation of all evaluated proteins, vectors and cells lines, as well as expression and purification products. The system also facilitates high-throughput workflows and incorporates various assays and analytics results that allow a systematic evaluation of protein production success.

REF-A144

HIGH THROUGHPUT SCREENING OF CRITICAL MONOCLONAL ANTIBODY QUALITY ATTRIBUTES

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KEY WORDS:
HTP SCREENING / SMALL SCALE PURIFICATION / TECAN AUTOMATION / ATOLL COLUMNS / MAB QUALITY

BACKGROUND AND NOVELTY:
With the advent of High-Throughput (HTP) techniques for cell line screening and process development comes a need for a scale down system that can assess the material generated for critical quality attributes. In order to assess a mAb for an acceptable quality profile there is the need to perform a variety of assays to determine different characteristics of the molecule. Common methods for purification and the assessment of product quality require a significant amount of starting material and time to perform the work both of which place a significant burden on available resource. This work describes how some of the methods and techniques have been adapted and applied to HTP Scale down systems. The benefits and limitations of these screening techniques will be presented.

EXPERIMENTAL APPROACH:
A wide range of characteristics can be considered important from both a functional and biophysical property perspective of a therapeutic molecule. Taking a number of different mAb characteristics in turn their relevance and application to an HTP screen was assessed. Orthogonal methods were evaluated and alternative HTP methods considered and evaluated for suitability.

RESULTS AND DISCUSSION:
While the need to fully characterise a cell line and resultant product exists from a regulatory perspective not all attributes and quality identifiers are necessary from an early cell line and process optimisation standpoint. This work shows how a Tecan robotic system, coupled with Atoll robocolumns can mimic mAb primary capture thereby reducing material requirements, time and cost. Furthermore orthogonal methods for glycosylation, charge profiling and aggregate can be replaced or, in some instances augmented for application as an HTP screen. This reduction in product characterisation time from 5 days to 1 day has enabled us to incorporate product quality evaluation earlier in our development process and to use this data to inform cell line and process selection.

REF-A145

CHARACTERIZATION OF MAB AGGREGATES IN A MAMMALIAN CELL CULTURE PRODUCTION PROCESS

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KEY WORDS:
PROTEIN AGGREGATION / MONOCLONAL ANTIBODY / MAMMALIAN CELL CULTURE / CHINESE HAMSTER OVARY

BACKGROUND AND NOVELTY:
The aggregation of monoclonal antibodies (mAbs) during production is a major concern. The presence of aggregates can reduce the therapeutic efficacy of the mAbs and trigger immunogenic responses upon administration. Higher molecular weight (HMW) aggregates can be removed during downstream processing (DSP), but this leads often to a reduction in process yields. Detection and inhibition of aggregates during the production process could reduce the burden on DSP and improve process yields. However, the size of aggregates ranges from small oligomers to visible particles and there is no single technique capable of measuring the broad range of aggregation problems. Therefore, we established methods to generate different types of aggregates and analytical tools to characterize the different aggregate species during a bioprocess in order to improve mAb production using mammalian cells.

EXPERIMENTAL APPROACH:
Aggregation of two mAbs produced in Chinese Hamster Ovary (CHO) cells was induced using heat stress, shaking stress, pH change and high salt concentrations. The presence of small aggregates was evaluated using size exclusion chromatography (SEC) and larger aggregates were characterized using dynamic light scattering (DLS) and turbidimetric analysis. The molecular weight of the aggregates was determined by SEC in combination with multi-angle light scattering (MALS) and refractive index (RI) analysis. Furthermore, the stability of the induced aggregates in cell culture medium and CHO cell culture supernatant was investigated.

RESULTS AND DISCUSSION:
All stress methods provoked aggregate formation. The mAbs showed formation of different aggregates using the different stress methods. The molecular weight as well as the hydrodynamic diameter of the HMW species was determined. Finally, the stability of the induced aggregates under cell culture conditions was evaluated and therefore the analytical methods can be applied to characterize mAb aggregate formation during the cell culture production process.

EVALUATION OF SINGLE-USE BIOREACTORS FOR PERFUSION PROCESSES

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KEY WORDS:
PERFUSION / SINGLE-USE / HARVEST / CHO

BACKGROUND AND NOVELTY:
Single-Use Bioreactors are now commonly used for in Process Development activities, as seeding production bioreactors or to produce drug substance. The advantages of these equipments has been well demonstrated over the last few years however,only a few data on their possible use in perfusion processes have been published. We have decided to evaluate these Bioreactors at different scale in combination with different harvest approach including Alternative Tangential Flow perfusion (ATF), Fibracell, microcarriers and Hollow fibers.

EXPERIMENTAL APPROACH:
The CellReady 3L Single-Use Bioreactor was used in a first series of experiments to evaluate the best process conditions. A number of repeats of each technology were evaluated in parrallel to determine final Process parameters and verify molecule quality (glycosylation, HCP, DNA, aggregates.....). The best culture and harvest conditions were then scaled-up to 50L Single-Use sytems to verify the feasibility at larger scale and then extrapolate to 200L scale for Production purpose.

RESULTS AND DISCUSSION:
The use of Single-Use technology is possible for perfusion processes and a number of variants can be used to do so. We have shown that we could keep the quality of the molecule in the defined specifications in a number of process conditions. These processes can be used at Process Dev. as well as Prodcution scale. The use of single-use versus stainless steel equipments pros and cons will be discussed in this study.

IMPROVEMENTS IN REDUCTION OF RESIDUAL DNA DURING PURIFICATION OF RECOMBINANT ANTIBODIES

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KEY WORDS:
RESIDUAL DNA / ANTIBODY PLATFORM / CAPRYLIC ACID / PURIFICATION / PRECIPITATION

BACKGROUND AND NOVELTY:
Therapeutic proteins used in clinical trials must fulfill several specifications. One major specification is the reduction of residual DNA (resDNA) in active pharmaceutical ingredients (APIs) to ≤ 100 pg/dose. For some recombinant antibodies the resDNA value after purification are too close to this specification. Therefore, we have investigated additional, alternative steps to reduce resDNA. We discovered that the use of caprylic acid reduced resDNA values significantly, if applied already in the cell culture supernatant.

EXPERIMENTAL APPROACH:
Diafiltration of cell culture supernatant and caprylic acid precipitation were checked for reduction of resDNA.

RESULTS AND DISCUSSION:
Comparison of diafiltration of cell culture supernatant at neutral pH and mild acidic pH displayed that only at mild acidic conditions a significant reduction of the DNA value could be obtained. DNA reduction in cell culture supernatant by precipitation with caprylic acid is dependent on the source of cell culture supernatant, on pH, incubation time and caprylic acid concentration. Precipitation of DNA with caprylic acid was found to be suitable for this purpose.

REF-A149

CONTINUOUS CELL CULTURES (AT 100 MILLION CELLS/ML AND BEYOND) USING XD® TECHNOLOGY COUPLED TO THE RHOBUST® EBA INTEGRATED CLARIFICATION AND PURIFICATION TECHNOLOGY

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KEY WORDS:
CONTINUOUS PROCESSING / VERY HIGH CELL DENSITIES / EXPANDED BED CAPTURE

BACKGROUND AND NOVELTY:
Over the past decade DSM Biologics has developed a number of breakthrough technologies in the field of mammalian cell culture process intensification and in the field of downstream processing unit operation process integration. Upstream with its highly intensified XD® cell culture process DSM has taken mammalian viable cell densities to extremes. Routinely operating over 150 million cells/m, record cell densities over 240 million cells/mL have been achieved and record titers of 27 g/L in the broth (and 40 g/L in the supernatant) have been reported and a consistent 5 - 10 fold titer boost compared to routine fed-batch operation. Downstream, with its Rhobust® technology, DSM has further developed expanded-bed technology to a point where it can deal in a very robust way with viscous and highly particle loaded feed streams, such as yeast-, E.coli-, but also mammalian XD® cell culture broths. In this paper Continuous Processing based on XD® and Rhobust® technologies is presented as the next logical step in process intensification and process integration in mammalian cell culture.

EXPERIMENTAL APPROACH:
A series of high cell density continuous cultures producing different IgGs and/or recombinant proteins have been performed. In XD® culture mode a hollow fiber retention system is used to keep both cells and product in the bioreactor, while fresh nutrients are continuously supplied to the bioreactor and spent medium is continously removed through the retention system. When operating XD® in a continuous mode, the cell density is increased to around 100 million cells/mL and subsequently kept constant by applying a cell bleed, in which the product is concentrated. Subsequently the cell bleed is applied to the Rhobust® EBA, where in a single step, cells, cell debris and the vast majority of the contaminants are removed and after elution clarified, concentrated and purified product becomes available.

RESULTS AND DISCUSSION:
Results of several continuous cultures in XD® mode including EBA harvesting are presented.

REF-A150

RISE OF THE MACHINES – AUTOMATING AN INTEGRATED PROCESS DEVELOPMENT WORKFLOW

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KEY WORDS:
AUTOMATION / UPSTREAM / DOWNSTREAM / ANALYTICS / ROBOTICS

BACKGROUND AND NOVELTY:
The modern day laboratory is evolving to accommodate a new workforce: automated systems. These systems are used to perform repetitive tasks with a high degree of accuracy and precision, allowing for increased sample throughput and process redesign. In this study, the use of automated systems to integrate upstream and downstream workflows in parallel with the automation of supporting analytics platforms is presented.

EXPERIMENTAL APPROACH:
Within process development, automation systems have been incorporated into the cell line construction, bioreactor evaluation, purification development and supporting analytics workflows. Each automation system is operated independently in what is known as an “island of automation” which allows for robustness, cost control and flexibility. Assessment from miniature to production scale has demonstrated that the automated systems can be used as predictive models of manufacturing processes.

RESULTS AND DISCUSSION:
The results of incorporating automation in to process development have included increase in throughput, timeline and cost reductions. Within the upstream process development workflow alone, five times the number of cell lines can now be assessed for productivity due to integration of liquid handling robots in the cell line screening processes. This is followed on by automated miniature bioreactor and purification systems, which is a more predictive model of the manufacturing process than classical approaches. In parallel, automated high-throughput analytical screening platforms have been introduced to perform product quality assays, such as analysis of glycan profiles and size variants, to reduce screening from days to hours. Within the workflow a robust process assessment using novel DoE designs as per regulatory guidelines ICH Q8-11 is performed. The integration of predictive models and supporting analytics into cell line development and purification processes enables selection of cell lines which produce suitable material (e.g. low aggregate, correct glycan profiles).

DEVELOPMENT OF A HIGH TEMPERATURE SHORT TIME (HTST) VIRAL LOAD REDUCTION MODEL AND PROCESS OPTIMIZATION FOR CELL CULTURE MEDIA TREATMENT IN AN ARMFIELD PILOT SCALE SYSTEM

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KEY WORDS:
VIRAL / HTST / RISK / MITIGATION

BACKGROUND AND NOVELTY:
High Temperature Short Time (HTST) is fast becoming an integral part of many biopharmaceutical companies' viral risk mitigation strategies. In order to identify optimal processing conditions, development of a small scale HTST model was required. A static tube immersion method was developed that allowed for small volumes of material to be processed while achieving a similar thermal heating profile as observed in the upscale unit. Evaluation of selected catalog product formulations were performed, with the resulting data set including viral load reduction, analytical and biolological performance, and productivity and product quality for a diverse set of products.

EXPERIMENTAL APPROACH:
A small scale tube immersion HTST method was developed where the thermal profile closely aligned with the pilot scale unit. The selected media formulations were spiked with a model of an industry relevant virus (Feline calicivirus). The spiked products were thermally processed at three pre-determined hold times and temperatures. Cell-based virus titrations were performed on the samples, and the viral log reduction was calculated. The operational parameters along with the associated viral log reduction data was transferred to the pilot scale unit. Following transfer of run parameters as defined in the static tube immersion system, selected processing parameters were run in the pilot scale system. Samples were collected from each pilot scale run and submitted for analytical, growth, and productivity testing.

RESULTS AND DISCUSSION:
All three process parameters run for each product achieved the target of greater or equal to four logs/mL virus reduction. Thermal profile for each run at static immersion tube scale aligned closely with upscale model. Minimal detrimental effects on cell growth and productivity were observed. Analytical results showed a decrease in concentration of various discrete components in some media. Additional testing can lead to development of media offerings optimized for HTST processing.

DEVELOPMENT OF AN ANTI-KAPPA AFFINITY BASED PURIFICATION PROTOCOL TO PRODUCE ANTI-INSULIN-RECEPTOR FAB FRAGMENTS FROM HYBRIDOMA CELLS

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KEY WORDS:
HYBRIDOMA / FAB FRAGMENT / PAPAIN / ANTI-KAPPA / AFFINITY CHROMATOGRAPHY

BACKGROUND AND NOVELTY:
Antibody fragments and smaller scaffolds may have a better tissue penetration compared to full IgGs. Drug delivery strategies like intranasal delivery is dependent on transport and/or diffusion processes, for both smaller molecules are of advantage. Insulin signalling has been shown to improve cognitive skills. An agonistic anti-insulin-receptor specific Fab fragment may allow modulation of insulin mediated effects in the brain. Hence, a protocol for generation and purification of anti-insulin-receptor Fab from hybridoma cells was established. Different novel anti-kappa light chain affinity resins based purification procedures were evaluated resulting in a novel rapid Fab purification protocol.

EXPERIMENTAL APPROACH:
The full IgG antibody was produced by hybridoma fermentation and captured by protein A affinity chromatography. Fab was generated by Papain. Undigested antibody and Fc fragments were removed by a second protein A capture step. The Fab fragment was polished comparing Capto L affinity medium (GE) and CaptureSelect anti-kappa (mur, BAC). Functional binding of the purified Fab to the human insulin receptor was studied by flow cytometry using fluorescently labeled anti-Fab antibody.

RESULTS AND DISCUSSION:
A novel easy to use protocol for generation and purification of Fab from hybridoma cells was successfully established. The full IgG was processed in two Fab fragments and the Fc using an antibody concentration of 2mg/mL incubated with 0.1 mg/mL papain at 37°C for 24 hours. Employment of the CaptureSelect anti-kappa affinity matrix revealed high yield at highest specificity resulting in >98 % pure protein. This allows a finalization of a rapid Fab purification protocol lacking the disadvantages of final SEC or IEX. After purification degradation has been observed in acidic buffers, therefore Fab fragments were stabilized by a shift to neutral pH. The binding functionality of the Fab fragment could be verified by extracellular staining of NIH3T3-A14 cells expressing the human insulin-receptor.

REF-A153

EVALUATION OF CRITICAL PROCESS PARAMETERS DURING UPSTREAM PROCESS DEVELOPMENT FOR MANUFACTURING OF RECOMBINANT ANTIBODY MIXTURES

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KEY WORDS:
UPSTREAM PROCESS DEVELOPMENT / ANTIBODY MIXTURES / MANUFACTURING

BACKGROUND AND NOVELTY:
Symphogen A/S has developed the proprietary Sympress™ single batch manufacturing platform for controlled manufacturing of recombinant antibody mixtures for therapeutic use in cancer and infectious disease. One of the most important objectives during process development of single batch manufacturing is to build in robustness and demonstrate proper control of a manufacturing process, thus ensuring that the antibody mixture product accomplishes consistently its target quality attributes (CQAs) and corresponding specifications. This is achieved partly through the cell line selection process, systematic process development, and characterization of the design space for each critical process parameter. During mapping of the upstream process design space, we have examined several different process parameters to quantify the impact on process performance in terms of productivity and product quality by a quality by design (QbD) approach. QbD approaches can lead to better process understanding and associated risk as-sessments through the use of process design of experiments (DOE). In the study provided here several different process parameters such as feeding strategy, seeding density and temperature have been studied for significant influences on CQAs by DoE. Thus via QbD and our Sympress™ expression platform technology various process parameter ranges were identified that yield acceptable CQA levels and that still provided operational flexibility for manufacturing. In conclusion, we were able to generate a target antibody composition and a robust production process showing high batch-to-batch consistency.

EXPERIMENTAL APPROACH:
see above, QbD and DoE.

RESULTS AND DISCUSSION:
see above.

REF-A154

RISE OF THE MACHINES – AUTOMATING AN INTEGRATED PROCESS DEVELOPMENT WORKFLOW

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KEY WORDS:
BIOPROCESS DEVELOPMENT / MAMMALIAN CELL CULTURES / MICROWELLS / ANTIBODY PRODUCTION

BACKGROUND AND NOVELTY:
The application of modern biotechnologies has significantly increased the use of antibodies and antibody fragments for human therapy. This is driven by the need to establish rational approaches to accelerate bioprocess development in producing cost effective and highly productive antibodies. This research described a microwell approach to obtain early process design data for the IgG production in Chinese Hamster Ovary (CHO) cells. Parallel microwell systems are employed to define the growth condition in batch and fed batch cultures.

EXPERIMENTAL APPROACH:
Nutrient feeding is achieved by bolus addition of concentrated feed resulted in higher IgG antibody titers compared to the batch culture. In addition, the shaken microwell system is equipped with optical probes to enable on-line monitoring of pH. Future work is aimed at automating the microwell system to achieve faster and scalable development of cell culture processes.

RESULTS AND DISCUSSION:
In terms of GS-CHO growth, the fed batch cultures resulted in a significantly higher viable cell concentration (VCC) than the batch cultures with 8.71 x 106 cells mL-1 and 7.69 x 106 cells mL-1 respectively. In terms of GS-CHO cell viability, fed batch cultures showed enhanced cell viability compared to the batch cultures with 82.5% of cell viability at the end of experiment. In the fed batch cultures, the feed supplemented restored the depleted glucose by 40%.

DEVELOPING NEXT-GENERATION CLARIFICATION PROCESSES OF RECOMBINANT PROTEINS FROM HIGH CELL DENSITY MAMMALIAN CELL CULTURES SYSTEMS

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BACKGROUND AND NOVELTY:

Increasingly high productivity cultures containing mammalian cells are placing a larger burden on traditional downstream clarification and purification operations due to higher product and impurity levels. Controlled flocculation of mammalian cell culture suspensions has been used as an alternative technology to enhance the clarification throughput and downstream filtration operations. While flocculation is quite effective in agglomerating cell debris and process related impurities, the resulting suspension is generally not easily separable by ordinary direct filtration methods. EMD Millipore aims to address this challenge by developing a proprietary Clarisolve® technology to improve primary and secondary direct depth filtration of pre-treated high cell density mammalian cell cultures.

EXPERIMENTAL APPROACH:

Cell culture harvest was pretreated by using either low pH induced flocculation or polyionic flocculants. The pH of the medium was adjusted from 6.9 to pH 4.5–5.0 using a 25% acetic acid solution. For the suspensions treated with flocculants, cell culture broth was treated with non-mammalian derived flocculant (chitosan), Polydiallyldimethylammonium chloride (pDADMAC) and proprietary EMD Millipore polymer flocculant (SmP E). Filtration experiments were done using Millistak® D0HC, X0HC and Clarisolve® 20MS, 40MS, and 60HX graded depth filters.

RESULTS AND DISCUSSION:

The filtration performance for the pH adjusted feed stream significantly improved with the 20MS filter type where throughput increased 4-fold to 260 L/m2 and a pool turbidity of 10 NTU over the D0HC control of untreated feed with D0HC. pDADMAC with an optimized dose of 0.08 % (weight/volume) with 40MS filter type demonstrated a throughput of 300 L/m2 and a pool turbidity of 3 NTU. In addition, chitosan, with an optimized dose of 0.08 % (weight/volume), with 40MS filter type demonstrated a throughput of 275 L/m2 and a pool turbidity of 5 NTU whereas SmP E with an optimized dose of 0.2 % (weight/volume) with 60HX filter type demonstrated a throughput of 310 L/m2 and a pool turbidity of 2 NTU. These data suggest optimizing media morphology and controlling the particle size distribution via flocculation result in the improved filtration performance as reflected by higher throughputs and lower post filter turbidities.

OTHER INFORMATION

We would like to thank Michael Peck, James Hamzik and KS Cheng from EMD Millipore for their excellent technical contributions on this project.

DESIGNING CLINICAL-GRADE INTEGRATED STRATEGIES FOR THE DOWNSTREAM PROCESSING OF HUMAN MESENCHYMAL STEM CELLS

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BACKGROUND AND NOVELTY:

During the past decade, stem cells have been the focus of an increased interest due to their potential in clinical applications, as a therapeutic alternative for several diseases. Within this context, human mesenchymal stem cells (hMSCs) have gained special attention due to their immune-modulatory and versatile characteristics, as well as in secreting bioactive molecules with anti-inflammatory and regenerative features. Although substantial efforts have been performed on the development of clinical-grade bioprocesses for the expansion of hMSCs in microcarrier-based stirred culture systems, the integration of downstream strategies that assure efficient cell-bead separation and consequent hMSC concentration and purification is required to deliver safe hMSCs to the clinic. Thus, our work has been focusing on the establishment of a scalable and clinical-grade bioprocess for the purification of hMSCs to support both autologous and allogeneic therapies.

EXPERIMENTAL APPROACH:

Our strategy consists in the design of an integrated downstream approach by combining membrane technology and novel chromatographic tools in a robust and cost-effective manner while compliant with good manufacturing practices (cGMP). Briefly, after hMSC expansion in microcarrier-based stirred culture systems, cells were harvested and separated from the microcarriers by filtration techniques. Different membrane technology tools were then evaluated aiming at concentrating and further purifying the final hMSC-based product.

RESULTS AND DISCUSSION:

Our results demonstrated that both steps yielded higher cell viabilities (over 90%) and recoveries (over 65%), without compromising hMSC quality. At the end of the process, hMSCs maintained their phenotype, proliferation capacity and multilineage differentiation potential. The purification strategy herein described can be easily adapted to a clinical/industrial setting, offering a scalable and disposable closed system alternative to the standard downstream processes currently used for the purification of hMSCs.

REF-B001

DEVELOPMENT AND EVALUATION OF A NEW, SPECIALLY TAILORED CHO MEDIA PLATFORM

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KEY WORDS:
CULTURE MEDIUM / CHO CELLS

BACKGROUND AND NOVELTY:
Today's biopharmaceutical industry is under increasing pressure considering cost efficient development with short timeframes starting from generation of producer cell lines to the final production process. Hence, the timescale for optimization of cell culture media is small, but it contains high potential for global process improvement. In this scope, our specially tailored media development platform, which allows a fast and reliable introduction of high-performance basis media and feeds, establishes new perspectives for an efficient process development.

EXPERIMENTAL APPROACH:
TeutoCell's new media platform was established based on various cell lines and expression systems (CHO-DG44/-GS/-K1). The platform medium is developed by a cyclic development strategy, utilizing theoretical and empirical formulation optimizations. The assessment of formulations was carried out in shaking flasks as well as closed-loop controlled 2 L bioreactor systems using standard conditions.

RESULTS AND DISCUSSION:
Presented results illustrate the direct adaption of cells from serum-containing basal medium or complex commercially available media to the chemically defined platform medium. Regarding adaption, during the development one focus was laid on easy protocols, allowing static, stirred or shaken cultivation systems, to meet the diverse requirements on customer's side. In addition, no supplementation of growth hormones or proteins is necessary for a performance comparable or superior to state-of-the-art media available on the market. A reduced quantity of components in the medium, more precisely less than 50 compared to up to 100 in common chemically defined media, facilitate an increased design space for the development of custom formulations. Up to now, this platform medium has successfully been utilized for a set of publically available and industrial cell lines. Generally, viable cell densities of more than 5 x 10E6 cells/mL were achieved in the platform medium, which underlines the broad potential of the technology.

REF-B003

INTRODUCING A NEW CHEMICALLY DEFINED MEDIUM AND FEED FOR HYBRIDOMA CELL LINES

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KEY WORDS:
HYBRIDOMA CELLS / MEDIA DEVELOPMENT

BACKGROUND AND NOVELTY:
Hybridoma technology was established in the 2nd half of the 20th century and in the view of current protein production it might seem outmoded. Despite, it is commonly used to produce monoclonal antibodies (mAbs) for R & D, clinical diagnostics or therapeutics and the demand for mAbs produced by hybridomas is still high. Nevertheless, compared to CHO, only a few serum-free hybridoma media are available and even less supplier for chemically defined products are on the market. The developed media and feed targets this gap to bring hybridoma processes to the next level.

EXPERIMENTAL APPROACH:
All cultivations were carried out using standard conditions well known in the art. Briefly, precultures and batch cultivations were performed in 125 mL and 250 mL Erlenmeyer flasks. Incubator conditions were set to 37°C, 5% CO2 and a relative humidity of 80%. For bioreactor cultivations closed-loop controlled 2 L benchtop systems were used with parameters set to 37°C, 40% DO and pH 7.1. Automated cell counting was performed by a Cedex. Quantitation of different antibodies was performed with Protein A HPLC.

RESULTS AND DISCUSSION:
The developed chemically defined medium and feed supports a direct utilization even for cells coming from serum-containing culture. Apart from that individual cell lines (e.g. NS0) might need additional supplementation like cholesterol, the development allows straight-forward usage for static or agitated uncontrolled culture systems, rocked disposable bags as well as stirred bioreactors. Several tested cell lines reached a maximum cell density above 4.0 x 10E6 cells/mL in batch and up to 1 x 10E7 cells/mL in fedbatch mode using shaking flasks. In addition, data for three different hybridoma cell lines are being presented, illustrating the results of closed-loop controlled batch, fedbatch and perfusion processes. In these cultures yields up to 500 mg/L of antibody in fedbatch mode and maximum viable cell densities of 20-30 x 10E6 cells/mL for perfusion (d = 2 vvd) were achieved.

TEMPERATURE DEPENDENCY OF IMMUNOGLOBULIN PRODUCTION IN NOVEL HUMAN PARNER CELL LINE

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KEY WORDS:
IMMUNOGLOBULIN / HYBRID / MAB / TUMOUR / TEMPERATURE

BACKGROUND AND NOVELTY:
A number of immunoglobulin (Ig) secreting human hybrid cell lines were created using one-on-one somatic cell hybridization of a rare human tumor infiltrating B lymphocyte and a cell of a novel human cell line (WTM), developed in house and described earlier. These hybrid cell lines secret various amounts of tumor-derived monoclonal antibodies (mAbs) of different specificities.

EXPERIMENTAL APPROACH:
Here we investigated an effect of mild hypothermic conditions on Ig production, cell growth and cell size in two experimental settings. In the first instance, three different hybrid cell lines each representing the highest, medium and lowest ranges of Ig productions, were subject to culture temperature drops from 37C to 36C, 35C or 34C for 168 hours with 24 hour data point intervals. In the second setting, the cell line with mAb production most susceptible to temperature drops was maintained at various temperatures below 37C (e.g. 36C, 35C and 34C) for at least 5 passages with each passage lasting 120 hours and the data taken at 24 hour intervals.

RESULTS AND DISCUSSION:
Whilst there was no observable effect of any of the short-term temperature drops on the cell growth and viability or the cell size in any of the three hybrid cell lines, the levels of Ig production consistently increased in all of them ranging between 67% and 320%, with Ig productivity peaking between 48 and 72 hours after the exposure to lower temperature. The most pronounced increase of Ig production was in the hybrid cell line initially producing the highest Ig level. In contrast with short-term temperature drop, prolonged exposure to mild hypothermic conditions (longer than 1 passage) led to progressive decrease in the cell size accompanied by gradual 10-20% gains of Ig production, which eventually leveled out after passage 4.Our results suggest that there might be different mechanisms responsible for the increase in Ig production in response to short temperature drop and prolonged hypothermia, which warrant further investigation.

ENHANCING CELL GROWTH AND ANTIBODY PRODUCTION IN CHO CELLS BY SIRNA KNOCKDOWN OF NOVEL TARGET GENES

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KEY WORDS:
SIRNA KNOCKDOWN / CELL LINE ENGINEERING / CHO CELLS / LENTIVIRAL GENE DELIVERY / MONOCLONAL ANTIBODY PRODUCTION

BACKGROUND AND NOVELTY:
Seven out of the ten top-selling biopharmaceuticals in 2011 are produced in Chinese Hamster Ovary (CHO) cells. This tremendous commercial interest makes the development and application of strategies for cell line optimization, like gene overexpression or knockdown to enhance cell specific productivity and cellular growth, highly interesting. In this work we investigated the knockdown effect of novel target genes by siRNA as a powerful tool for CHO cell line engineering.

EXPERIMENTAL APPROACH:
siRNA knockdown of different target genes was achieved by stable introduction of an shRNA vector via lentiviral gene delivery into the antibody producing model cell line CHO DP-12. The novel target genes Mcm5, JNK, Bad and Set were chosen from previous CHO cDNA microarray results and based on pathway analysis of apoptosis, cell cycle or histone modification. Successful mRNA knockdown was verified by quantitative real-time PCR. Effects on growth and antibody productivity were evaluated in batch and fed-batch shaker cultivations.

RESULTS AND DISCUSSION:
Knockdown of apoptosis mediators JNK and Bad resulted in an increase in viable cell density by 23 % and 43 %, respectively, compared to a control culture carrying an empty transfer vector. For cells with a knockdown of the cell cycle associated gene Mcm5, an enhancement in peak viable cell density of 46 % in combination with a prolonged production phase by two days was achieved. Due to the higher cell densities, product titers were elevated as well. Knockdown of Set, which plays a role in histone modification, did not alter growth and productivity compared to control cells. For the most successful target (patent application in preparation), maximum viable cell density and antibody concentration increased by 123% and 159%, respectively. In conclusion, our results indicate that siRNA mediated gene knockdown is a promising strategy for cell line engineering.

REF-B006

OVEREXPRESSION OF SERPINB1 IN CHINESE HAMSTER OVARY CELLS INCREASES RECOMBINANT IGG PRODUCTIVITY AND CELL GROWTH

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KEY WORDS:
HOST CELL ENGINEERING / SERPINB1 / CHO CELLS / CELL GROWTH / GENE OVER-EXPRESSION

BACKGROUND AND NOVELTY:
We report the discovery and validation of a novel CHO cell engineering target, Serpinb1, that has significant effects in enhancing cell growth and IgG productivity. Previously, we performed transcriptomic studies using cDNA microarrays and compared cultures with IgG heavy and light chain (HC and LC) transiently knocked down versus cultures with non-targeting siRNA. The transcription level of Serpinb1 (serpin peptidase inhibitor, clade B, member 1), a member of the serine protease inhibitor superfamily, was up-regulated 2 fold post HC and LC siRNA transfection.

EXPERIMENTAL APPROACH:
A proprietary lentiviral vector was employed to overexpress the Chinese Hamster Serpinb1 in a CHOZn Glutamine Synthetase (-/-) recombinant IgG producing line. A plasmid expressing Serpinb1 was transfected to the CHOZn GS (-/-) host cell line to create three stable pools at various selection levels.

RESULTS AND DISCUSSION:
The lentivrial transduction led to a stable pool with 4.2-fold SERPINB1 coverexpression. The pool's peak viable cell density and IgG volumetric productivity in fed-batch increased 1.3- and 2.0 -fold, respectively, compared with the non-transduced control. Over 60 single-cell clones isolated from the plasmid pools were characterized for their SERPINB1 expression and exponential phase growth rate in fed-batch cultures. We observed that some clones with the highest SERPINB1 expression derived from the plasmid stable pools had decreased exponential phase growth rate, whereas the clones with moderate SERPINB1 overexpression demonstrated increased growth rate. Selected clones with varied SERPINB1 over-expression levels were evaluated for their IgG transient and stable expression capabilities using GS selection. We conclude that Serpinb1 is a suitable cell engineering target for biopharmaceutical production, and manipulating Serpinb1 expression can lead to increased cell growth and recombinant IgG productivity. This is among the first successes in applying "-omics" findings to CHO host cell engineering.

REF-B007

EFFECT OF RECOMBINANT PROTEIN LOAD ON THE ACTIVATION OF THE UNFOLDED PROTEIN RESPONSE (UPR) IN CHINESE HAMSTER OVARY (CHO) CELLS

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KEY WORDS:
ER STRESS / SECRETORY BOTTLENECK / ERYTHROPOIETIN / CHINESE HAMSTER OVARY / UNFOLDED PROTEIN RESPONSE

BACKGROUND AND NOVELTY:
The production of a recombinant protein (RP) in mammalian cells is regulated at multiple steps (1-4). Several laboratories have illustrated that RP production can be limited at one or more stages of the secretory pathway (5-7). This limitation has been characterised by the activation of the unfolded protein response (UPR) pathway and the presence of misfolded proteins in cells producing high amounts of RP (6, 7). The aim of this work is to profile of UPR component status for recombinant CHO cell lines which differ in terms of specific productivity. This data will be used to define settings of UPR gene expression that are associated with improved capacity to support complex protein production.

EXPERIMENTAL APPROACH:
A series of clones of Chinese Hamster Ovary (CHO) cells, expressing erythropoietin (EPO), were characterised in terms of growth and productivity (ELISA). Structural variant profiles of intracellular and secreted EPO were determined by western blot. UPR target genes expression during batch culture were measured by RT-qPCR.

RESULTS AND DISCUSSION:
Recombinant CHO cell lines exhibited a 5-fold range of EPO specific productivities (Qp) between the amplified and non-amplified cell lines. Increased Qp was correlated with an increased intracellular content of EPO and the presence of high molecular weight intracellular EPO species were observed in the highest producing cell line. Despite the presence of abnormal intracellular EPO variants in cell lines with the highest Qp, there was no enhancement to the activation of the XBP-1 arm of the UPR and other genes profiled in this study showed similar dynamics for cell lines during batch culture. The accumulation of incompletely glycosylated EPO inside cells suggests that after passing a key quality assessment step fully glycosylated EPO is quickly secreted. This key post-translational modification presents a limiting step for effective EPO secretion and offers a potential target for cell host selection or genetic engineering.

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OTHER INFORMATION
I would like to thank all the members of the Dickson Lab, especially Zeynep Duman for recombinant cell clones that initiate my study and for sharing her experience.
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DEVELOPING NEXT-GENERATION CLARIFICATION PROCESSES OF RECOMBINANT PROTEINS FROM HIGH CELL DENSITY MAMMALIAN CELL CULTURES SYSTEMS

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KEY WORDS:
PER.C6® / CELL CYCLE / APOPTOSIS

BACKGROUND AND NOVELTY:
PER.C6® is a human cell line designed for virus production, which was immortalized by transformation with adenoviral E1A and E1B genes. Expression of E1A is known to inhibit negative regulators of cell cycle and E1B protein function analogously to an apoptosis inhibitor. As changes in cell cycle and apoptosis are likely to affect cell's ability for viral infection and propagation, the study of these parameters in PER.C6® cultures is essential to develop optimum virus production processes.

EXPERIMENTAL APPROACH:
Cell cycle distribution and apoptosis were measured in batch and perfusion PER.C6® cultures using flow cytometry. Propidium iodide was used to measure cell cycle distribution. Three methods were used to measure apoptosis: staining of externalized phosphatidylserine (PS) using annexinV, staining of activated caspases using a fluorochrome-conjugated inhibitor of caspases, and staining of fragmented DNA using BrdU incorporation and specific fluorescent labeling. 7-ADD was used to stain dead cells with a permeable membrane. Significant cell death occurred in 14-days batches, when the main carbon sources were depleted. Apoptosis was initially not detected by the annexinV staining. However, activated caspases were detected after 6 days, suggesting that apoptosis occurred in batch. In perfusion, where the required nutrients were constantly supplied, no significant cell death or induction of apoptosis occurred, showing that the cultures were maintained in healthy conditions. At the end of batches, the portion of cell in S phase increased drastically and the one in G0/G1 decreased. In perfusion, cell cycle distribution was stable until 10 days, when a similar trend as the end of batch was observed.

RESULTS AND DISCUSSION:
Significant cell death occurred in 13-days batch cultures when the main carbon sources were depleted, without a change in the basal apoptotic level measured throughout the batch. Similarly, when perfusion cultures reached over 100x10^6 vc/mL and the required nutrients could not be supplied, trypan-blue based viability decreased significantly but apoptosis did not increase. Cell cycle distribution for these perfusion cultures was stable until 10 days, when the portion of cells in S phase increased and in G0/G1 phase decreased. This is the first research showing the limited occurrence of apoptosis and the increase of cells in S phase at the end of a PER.C6® perfusion culture. Our data are in accordance with the theoretical effect of immortalization by the E1A/B system, which promotes the entry into the cell division cycle and inhibits apoptosis. Other pathways for cell death (e.g. necrosis) may be more important than apoptosis in PER.C6® and in other E1A/B immortalized cell lines.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
This is the first research describing apoptosis and cell cycle distribution in PER.C6® batch and perfusion cultures. Our data are in accordance with the theoretical effect of immortalization by the E1A/B system, which inhibits apoptosis when nutrients are in excess and promotes the entry into the cell division cycle.

STRATEGIES FOR CLONE DETECTION, SELECTION AND ISOLATION IN PERC.6 CELLS – CASE FOR REBMAB100

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KEY WORDS:
MONOCLONAL ANTIBODY / PERC.6 CELLS / CLONEPIXFL / LEWISY / EPITHELIAL CARCINOMA

BACKGROUND AND NOVELTY:
An efficient monoclonal antibody (mAb) cell line development requires improvements in clone detection and screening. To compare with the high time-consuming limiting dilution cloning (LDC) we have implemented the ClonePixFL (CP-FL), an automated system for high throughput cell based research. Selecting a stable and productive clone weighs heavily for the success of a product and process development can improve it further. We have used both automated clone picking and LCD to generate clones for the Rebmab100 mAb recognizing the LewisY antigen highly present on the surface of many epithelial carcinomas. We have also combined these two approaches with high throughput screening assays for early selection of highly productive clones.

EXPERIMENTAL APPROACH:
Starting from the heterogeneous transfected populations, 8 6-wells microplates were seeded with cells in semi-solid medium for PerC.6 growth. A total of 845 colonies were picked, from which 225 were transferred to 24-wells microplates. For the LCD, 50 96-wells microplates were seeded with cells in liquid medium, and 261 colonies transferred to 24-wells microplates. Sequential steps were transfer to 6-wells microplates, T-flasks and shaker flasks, keeping the best clones based on cell growth and productivity.

RESULTS AND DISCUSSION:
Both procedures proved efficient for generating high productive and stable cell clones. 31 clones adapted to suspension cultures were selected for further analysis, 15 originated from LDC and 16 from CP-FL. The small scale fed-batches resulted in 12 clones with titers ranging from 1.3 to 3.0g/L. The automated picking has the advantages of less labor and time, allowing the chance of picking clones that would not grow isolated in LCD. From a total of 4800 wells seeded in LDC, 261 colonies were obtained and 225 colonies out of 845 wells seeded with cells picked by CP-LF, representing 5.4% and 26.6% efficiency, respectively. Further analysis is ongoing to determine which clones show superiority in large scale.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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OTHER INFORMATION
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REF-B010

THE BENEFITS OF SINGLE CELL CLONING FOR MAB PRODUCTION: STABILITY, PRODUCTIVITY AND PRODUCT QUALITY

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KEY WORDS:
FACS / SINGLE CELL CLONING / CRITICAL QUALITY ATTRIBUTES / CELL LINE IMPROVEMENT

BACKGROUND AND NOVELTY:
New cell lines fit for the manufacture of biopharmaceutical products should ideally exhibit many critical quality attributes. The cell line should be: stable, demonstrate high productivity and produce material with an acceptable quality profile. In some instances not all of these attributes are met to a satisfactory degree from a single round of clone selection. In these case studies we describe the benefits of Fluorescence-Activated Cell Sorting (FACS) to enhance all three of these attributes, improving either stability, productivity or mAb quality.

EXPERIMENTAL APPROACH:
Following generation of lead antibody-producing CHO cell lines for a variety of projects lead lines were further subjected to a FACS procedure. Parent lines were FACS sorted according to standard in-house protocols and resultant daughter clones evaluated using standard in-house screening techniques for cell line stability, productivity and antibody quality.

RESULTS AND DISCUSSION:
An unstable Master Cell Bank (MCB) of a mAb producing cell line was single cell sorted using FACS. The resultant daughter clones were evaluated for both productivity and cell line stability and the lead clones showed improved cell line stability compared to the parent. FACS was also used to resort a second cell line originally generated by dilution plating and the resulting lead single cell clone was shown to have an increased specific productivity (SPR) compared to the parent. Finally a lead clone for a third project was FACS sorted and resulting clones screened for a more favourable profile in comparison to the parent, the lead daughter clone was found to exhibit a significantly lower aggregate level. In all instances FACS treatment resulted in a marked improvement of a critical quality attribute when compared to the pre-FACS parent.

REF-B011

EFFECTS OF MODIFICATION IN SIGNAL PEPTIDE ON PRODUCTION OF RECOMBINANT ERYTHROPOIETIN FROM RECOMBINANT CHO CELLS

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KEY WORDS:
SIGNAL PEPTIDE / ERYTHROPOIETIN / PROTEIN SECRETION / RECOMBINANT PROTEIN PRODUCTION / PRODUCTIVITY INCREASE

BACKGROUND AND NOVELTY:
Various secretory proteins are synthesized as precursors with additional N-terminal signal peptide. The signal peptide is cleaved off by signal peptidase once it has served its purpose of targeting the protein to, and importing it into, the ER. Recently, recombinant DNA research was used to study signal peptide and made it possible to show the efficient activity of a proposed signal peptide by fusing it to another protein.

EXPERIMENTAL APPROACH:
In this study, signal peptide of human erythropoietin was replaced with the signal peptide of human IL-2, then the hydrophobic region of the signal peptide was modified and its effect on the production of the target protein (erythropoietin) was evaluated. The nucleotide sequence of modified signal peptide was transported directly into the upstream of the 5' end of the human erythropoietin gene by performing one round of amplification with T4 DNA polymerase. The gene of target protein with modified signal peptide was transiently expressed in CHO cells and quantification of protein secretion was evaluated.

RESULTS AND DISCUSSION:
As a result, we could observe significantly increased protein secretion up to 4 times high by modifying the native signal peptide, in particular the hydrophobic region. This observation is believed to be applied to improve the productivity of recombinant therapeutic proteins from animal cells.

APPLYING LASER EMISSION AIDED PROCESSING (LEAP) TO SCREEN MUTANT CHO LIBRARIES

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KEY WORDS:
CHO / MUTATION / LEAP / BIOPROCESSING

BACKGROUND AND NOVELTY:

Increasing the amount of desired recombinant proteins is central to making mammalian bioprocressing more efficient. At the the Novo Nordisk Foundation Center for Biosustainability (CFB) we are working towards improving the yields of Chinese Hamster Ovary (CHO) cells by applying novel Laser Emission Aided Processing (LEAP) based screening methods and identifying mutations and clones that are beneficial.

EXPERIMENTAL APPROACH:

The LEAP machine is a tool that can be used to perform high throughput screening of individual cells ability to secrete a desired product, followed by single cell selection by laser ablation of undesired cells. By combining this technique with mutagenesis strategies, we are screening libraries of CHO mutants and identifying high producers as a clonal population. Once desired mutant clonal populations have been isolated, the mutation site and expression location will be identified and the effect verified through targeted knockouts. This technique will enable us to identify genes and phenotypes that enhance the productive capacity of the CHO host cell line going forward.

RESULTS AND DISCUSSION:

To enable this screening method, selection assays are being developed and verified for a number of model products. Preliminary results demonstrate the feasibility of the method using model secreted proteins as we continue to work towards identifying beneficial mutations for improving protein production from CHO cells.

ENHANCED CELL-SPECIFIC IGG PRODUCTIVITY IN THE PRESENCE OF HYDROLYSATE IS ASSOCIATED WITH SPECIFIC METABOLIC PROFILES

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KEY WORDS:
CHO / CELL-SPECIFIC PRODUCTIVITY / METABOLIC PROFILE / HYDROLYSATE / OSMOLALITY

BACKGROUND AND NOVELTY:

Chinese hamster ovary (CHO) cells are hosts for the production of large, complex biopharmaceuticals, with correct post-translational fidelity. However, we lack a comprehensive understanding of how environmental changes (e.g. medium composition) translate into phenotypic status at the molecular and cellular level, within the context of genetically diverse cell populations. Hydrolysates have previously been shown to improve IgG yield in an exemplar CHO fed-batch process and were associated with a metabolic shift to lactate consumption. In this study, we applied Gas Chromatography tandem Mass Spectrometry (GC-MS) metabolic profiling to define the relationships between culture phenotype and metabolic profile of an exemplar hydrolysate fed-batch IgG-CHO bioprocess.

EXPERIMENTAL APPROACH:

A proprietary IgG-expressing CHO cell line was cultured in shake flasks. Industrial hydrolysate was optionally added after 5 days. Samples from hydrolysate-fed and unfed cultures were obtained for cell enumeration, viability assessment, omsolality, pH, cell cycle analysis, total protein determination, IgG titres, oxygen consumption rate and metabolite profiling. GC-MS analysis on methanol-deproteinized medium samples was performed as described by Sellick et al (2009).

RESULTS AND DISCUSSION:

Hydrolysate addition increased culture osmolality, and produced a prolonged phase of proliferative arrest (in G1/G0) with enhanced cell-specific productivity compared with unfed controls. Proliferative arrest was accompanied by a prominent population of enlarged cells, with greater cell protein content. Hydrolysate addition also extended culture duration, and increased final product yield. The metabolic profile altered after hydrolysate addition towards a highly oxidative metabolic state in which lactate and alanine were consumed in addition to glucose. We provide insight into the mechanisms linking hydrolysates to greater yield in CHO cell bioprocesses, and address important general facets of metabolism, biomass and productivity optimization.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

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OTHER INFORMATION

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REF-B014

DEVELOPMENT OF A CHEMICALLY DEFINED CULTIVATION AND TRANSFECTION MEDIUM FOR HEK CELL LINES

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KEY WORDS:
TRANSFECTION / CHEMICALLY DEFINED / MEDIUM DEVELOPMENT / TRANSFECTION EFFICIENCY

BACKGROUND AND NOVELTY:
In the process of generating a production cell the introduction of the gene of interest into the host cell can be performed by various physical, chemical or biological methods. Because of the greater scalability compared to physical methods and no safety concerns or restrictions that are associated with the use of viral systems, a transfection using chemical methods is of great interest. Considering the upscaling to gram yields, a culture medium that allows both, transfection and production is required. By this, processes will no longer being limited by media exchange prior transient transfection.

EXPERIMENTAL APPROACH:
Transfection was performed according to standard protocols described in the literature. Briefly, 5x10E6 cells/mL were transfected with 2 pg DNA/cell and 25 kDa PEI. All cultivations were carried out using shake flasks with standard conditions well known in the art. Automated viable cell counting was performed by a Cedex. Furthermore, the quantities of components like glucose, lactate, amino acids, salts and vitamins in the supernatant were measured. Based on this information, single ingredients or groups of components from the basal formulation were screened for their influence on transfection efficiency. To evaluate the effect of cellular proteins in conditioned medium they were separated by chromatography and analyzed via MALDI-TOF-TOF mass spectrometry.

RESULTS AND DISCUSSION:
The screening of basal medium components exhibited no significant influence on transient transfection efficiency of HEK cells (overall efficiency of 80% +/- 15%). In contrast, depending on the level of conditioning the presence of proteins in the supernatant of these media reduced transfection efficiency up to 100%. This is currently under further investigation. In addition, the optimization of the basal transfection medium regarding growth of different HEK cell lines and promoting single cell growth without using anti-clumping agents, which are known to interfere with cell transfection, is in focus of our work.

REF-B015

GOLGI ENGINEERING OF CHO CELLS BY TARGETED INTEGRATION OF GLYCOSYLTRANSFERASES LEADS TO THE EXPRESSION OF NOVEL ASN-LINKED OLIGOSACCHARIDE STRUCTURES AT SECRETORY GLYCOPROTEINS

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KEY WORDS:
GOLGI ENGINEERING / RECOMBINANT BIOTHERAPEUTICS / CHO CELL

BACKGROUND AND NOVELTY:
Therapeutic activity of glycoproteins is substantially influenced by Asn-linked oligosaccharides. Biotechnologically used mammalian host cell lines are limited by their intrinsic glycosylation machinery and produce recombinant proteins with host specific glycoforms. To express glycoproteins with tailored glycosylation characteristic, we have constructed novel CHO cell lines that co-express a glycosylated secretory reporter protein together with the membrane-bound form of human GlcNAc transferase III (GnT3), 1,3-fucosyltransferase VII (FT7), a variant of 1,3-fucosyltransferase VI (varFT6, targeted to an early Golgi compartment) or the 1,4-GalNAc transferase 3 (B4GalNT3).

EXPERIMENTAL APPROACH:
Plasmids encoding the glycosyltransferases GnT3, FT7, varFT6 and B4GalNAcT3 were transfected into CHO suspension cells that stable express a monoclonal antibody (mAB) which comprises two Asn-linked glycosylation sites at the Fab and Fc domain, respectively. The N-glycans released from the purified products of genetically engineered CHO cells were subjected to sensitive oligosaccharide analysis methods (HPAEC-PAD, 2AB-HPLC, ESI-MS/MS) and compared to the mAB glycans from the wild type CHO cell line.

RESULTS AND DISCUSSION:
mAB oligosaccharides from wild type cells demonstrated the presence of neutral diantennary N-glycans at the Fc fragment and 2-3Neu5Ac-linked diantennary N-glycans at the Fab fragment. Structures from GnT3 CHO cells were considerably decorated with bisecting GlcNAc at both glycosylation sites. In contrary, co-expression of varFT6 or FT7 resulted in the exclusive attachment of 1-3Fuc residues to N-glycans at the Fab fragment generating Lex and sLex determinants. Finally, B4GalNT3 activity yielded structures terminated with the very uncommon LacdiNAc motif. Thus, our engineered cell lines can be successfully used for the production of recombinant proteins with novel glycosylation characteristics that might improve therapeutic in-vivo properties (e.g. tissue addressing, stability).

REF-B017

FED-BATCH PROCESS DEVELOPMENT FOR GLYCOPROTEIN PRODUCTION: EXPLOITING THE ALTERED METABOLISM OF HEK-293 CELLS EXPRESSING PYRUVATE CARBOXYLASE

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KEY WORDS:
HEK-293 / PYRUVATE CARBOXYLASE / FED-BATCH / METABOLISM / GLYCOPROTEIN

BACKGROUND AND NOVELTY:
Fed-batch cultivation is the most prominent mode used in industrial cell culture processes, since it can alleviate early nutrient limitations encountered in batch. However, the accumulation of lactate and ammonia is often the critical factor limiting fed-batch cultures and several cellular engineering approaches have been proposed to minimize the formation of these waste metabolites. We have shown previously that overexpressing the yeast pyruvate carboxylase 2 (PYC2) gene in HEK-293 cells can significantly alter their central metabolism towards a more efficient utilization of nutrients and a concomitant reduction in by-product formation. In this work, we demonstrate that the unique phenotype of PYC-expressing cells can be exploited to yield significant process improvements through the use of concentrated feeds.

EXPERIMENTAL APPROACH:
The study was conducted using two recombinant cell lines; a parental HEK-293 cell line stably expressing high levels of recombinant interferon-a2b and a clone overexpressing the PYC2 gene. Their kinetics of growth, nutrient consumption and waste formation were determined in batch and fed-batch cultures. For the latter, concentrated solutions consisted in both commercially available formulations and custom feeds containing amino acids and vitamins.

RESULTS AND DISCUSSION:
In batch, the PYC-expressing cells yielded much lower lactate concentrations and exhibited a 2-fold increase in maximum cell density over parental cells. In fed-batch cultures, the addition of concentrated nutrient solutions allowed to further increase the maximum cell density by 40 % and to extend the culture longevity by 60 %, without causing a significant increase in waste metabolite accumulation. Similar improvements were obtained with both commercial and custom-made concentrated solutions, demonstrating that the favorable metabolism of these cells make them particularly well-suited for fed-batch operation. In contrast, the same feeding protocols only led to minor improvements in the case of parental cells.

REF-B018

METABOLIC PROFILING OF RECOMBINANT NS0 CELL LINE DURING HIGH CELL DENSITY PERFUSION CULTURES

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KEY WORDS:
HIGH CELL DENSITY / PERFUSION PROCESS / NS0 NON GS CELL LINE

BACKGROUND AND NOVELTY:
High cell concentration and productivity can be achieved in perfusion processes.The successes of maintaining a good physiological microenvironment with high cells concentration is mainly reached by continuous addition of specific nutrient to the medium and removal of metabolic by-products.In this way understanding mechanisms underlying enzymatic control of energetic pathways and metabolites transporters expression in recombinant cell lines allow to determine the medium components that primarily influence in culture growth and specific productivity.However the study metabolic behavior of recombinant cells at high cell densities and transient stages during the culture process up to reach these densities is not well understood yet,especially for non GS NS0 cells.This work is an attempt to contribute to this understanding.

EXPERIMENTAL APPROACH:
NS0 cell line expressing an IgG1 humanized Mab was cultured in perfusion mode. An in house developed serum free medium supplemented with amino acids, vitamins, iron salt, ethanolamine, cholesterol, phospholipid and insulin was used. Sample from different stages of cell cultures were analyzed.Cell cycle, intracellular IgG and apoptosis methods were performed using a FACScan. Enzymatic activities of G6PDH, LDH, PYK, Glutaminase and ALAT, ADP/ATP ratio were measured. GLC, GLN and LACT were determined.

RESULTS AND DISCUSSION:
The content of cholesterol, total lipids, glucose and glutamine was lower at higher cell densities.The enzyme activity of G6PDH, ALAT, PYK and Glutaminase was higher in cultures at low cell densities; meanwhile a reduced specific production rate in parallel with increased frequency of cells in GoG1 phase was detected. In high cell density we observed a reduced glucose and glutamine consumption; however the ADP/ATP ratio was diminished. The frequency of apoptotic cells was still stable during the whole culture. Obtained results demonstrate that changes in the synthesis of macromolecules and in the energy metabolism of the cell line with increased cell densities in the culture.

REF-B019

CHO STARTER CELL LINES FOR MANUFACTURING OF PROTEINS WITH PRE-DEFINED GLYCOPROFILES

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KEY WORDS:
CHO / GLYCOENGINEERING

BACKGROUND AND NOVELTY:
Targeted modification of glycan structures of monoclonal antibodies produced in mammalian cell culture is a highly attractive approach for enhancing their pharmacokinetic and dynamic properties. Industrial CHO cell lines have several deficits in their glycosylation pattern, at least for some applications, like high fucose content (corresponding to a low ADCC profile) and low galactosylation and sialylation levels (proposed decreased pharmacokinetics). To overcome these drawbacks or to make them adjustable, we evaluated a whole set of glycomodulating enzymes for CHO-K1 und CHO-DG44 cell lines.

EXPERIMENTAL APPROACH:
First, we established a well characterized set of starter CHO cell pools each containing different glycan modulators. Single cell cloning was used to generate cell clones for intensive testing of clonal properties, e.g. growth profile and modulator expression level with corresponding glycoprofiles. Candidates with matching properties were pooled again to generate genetic diversity required to obtain the highest producer lines for a wide spectrum of products.

RESULTS AND DISCUSSION:
Here we show case studies demonstrating how this technology platform is performing under different conditions. The available set of data will give an in depth view of this technology, making it easier to develop glycoproteins with predefined glycoprofiles.

REF-B020

STRATEGIES FOR MITIGATING AND CONTROLLING PROCESS, MEDIA VARIABILITY, AND PRODUCT QUALITY ISSUES DURING UPSTREAM DEVELOPMENT OF AN IGG4 MONOCLONAL ANTIBODY

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KEY WORDS:
LACTATE / IGG4 / COLORATION / REDUCTION / ANTIBODY

BACKGROUND AND NOVELTY:
This presentation describes the optimization of a CHO (Chinese Hamster Ovary) production process for an IgG4 monoclonal antibody (MAb), and the strategy employed for resolution of multiple, critical process issues encountered during development. The issues included: 1) drug substance(DS) coloration, 2) variable metabolic shifts and elevation of lactate, 3) growth medium lot-to-lot variability, impacting cell metabolism, yield, and process scale translation, 4) in-process MAb reduction, and 5)large fluctuations in product yield (up to 85%) and viable cell growth. The effects were amplified due to the hyper-sensitivity of the production CHO bank to discrete fluctuations in sparging, osmolality, dissolved carbon dioxide, pH, lactate, and ammonia. Root causes for the issues were identified, and optimized feed, harvest, and control strategies were designed to completely restore process robustness regardless of media lot or production scale. This case study is a model for the resolution of multiple complex process development challenges, resulting from scale-up, tech transfer, media variability, and cell line sensitivity. The case study also provides insight into approaches for troubleshooting critical process issues efficiently without impacting product development timelines and product quality.

EXPERIMENTAL APPROACH:
A statistical design of experiments (DOE) and process scale-down approach at the 2-L and 20-L bioreactor scales was employed to address the performance variability issues.

RESULTS AND DISCUSSION:
Performance fluctuations were linked to growth medium lot variability and the variable levels of a single component in the medium. Similar control of the DS coloration was achieved through control of three media components. Optimized gas sparging and harvest strategies were employed to resolve the remaining issues of reduction and scalability. Critical process parameters were identified through DOE process robustness studies to modulate the impact of other factors on yield and product quality.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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SHEFF-PULSE AN ANIMAL COMPONENT FREE COMPLEX FEED SYSTEM THAT ENHANCES GROWTH AND PRODUCTIVITY OF CHO-K1 AND CHO DG44 CULTURES

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KEY WORDS:
COMPLEX FEED SYSTEMS / CHO CELLS / UPSTREAM PROCESSING / SHEFF-PULSE / MEDIA SUPPLEMENT

BACKGROUND AND NOVELTY:
Media development strategies are a constant area of focus for the Biopharmaceutical industry with simplification, predictability and consistency of upstream processes a major goal. This study describes an ACF feed system, Sheff-Pulse, which exploits existing synergies among plant derived hydrolysates, yeast extracts and recombinant proteins while focusing on the specific nutritive needs of common production CHO cell lines. The synergy between these ingredients is a powerful tool to increase cell culture based biopharmaceutical process yields and reduces media-related cost. The Sheff-Pulse feed system was designed to combine with a wide range of basal media.

EXPERIMENTAL APPROACH:
In order to evaluate the applicability of the complex feed system in CHO cell lines, Sheff-Pulse was screened in a CHO-K1 cell line expressing SEAP and a CHO DG44 dhfr- cell line expressing IgG. Multiple rounds of fed-batch shake flask experiments were performed in both CHO models in classical basal media or commercially available chemically defined (CD) media. Viable cell density and nutrient profiles of Sheff-Pulse supplemented shake flask cultures were generated and compared to cultures fed with a series of commercially available feed supplements. Media supernatant was also collected to assess the effect of each feed strategy on either SEAP or IgG production. CHO DG44 fed-batch shake flask results were then verified in 1L DASGIP bioreactors.

RESULTS AND DISCUSSION:
When supplemented into a cost-effective DMEM basal media, the Sheff-Pulse feed system demonstrated an equivalent or enhanced ability to improve overall CHO-K1 culture performance as compared to a series of commercially available, chemically defined feed supplements. Application of the Sheff-Pulse feed system to a CHO specific, chemically defined basal media demonstrated performance improvements in both CHO-K1 and CHO DG44 models. Results demonstrate that Sheff-Pulse ACF feed system is an effective and efficient alternative to other commercially available feed supplements.

USE OF SHEFFIELD BRAND CHEMICALLY DEFINED SUPPLEMENTS SHEFF-CHO CD FOR THE IMPROVEMENT OF CELL GROWTH AND RECOMBINANT PROTEIN TITER

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KEY WORDS:
CHEMICALLY DEFINED SUPPLEMENTS / CHO CELLS / CELL GROWTH / RECOMBINANT PROTEIN TITER / BIOPHARMACEUTICAL PRODUCTION

BACKGROUND AND NOVELTY:
There has been a growing trend towards the use of Chemically defined media and supplements in the biopharmaceutical industry in an effort to improve product consistency. To meet this trend Kerry has developed CHO specific CD supplements with the intent to increase biopharmaceutical process yield, improve culture health and reduce media-related cost.

EXPERIMENTAL APPROACH:
A CHO-K1 cell line modified to produce secreted alkaline-phosphatase (SEAP) and CHO-DG44 dhfr- antibody producing cell line was used to develop the CD supplements. Multiple rounds of fed-batch shake flask experiments were performed in both CHO models in classical basal media or commercially available chemically defined (CD) media. Viable cell density and nutrient profiles of the Sheff-CHO CD supplemented shake flask cultures were generated and compared to cultures fed with a series of commercially available feed supplements. Media supernatant was collected to assess the effect of each feed strategy on either SEAP or IgG production. CHO DG44 fed-batch shake flask results were then verified in 1L DASGIP bioreactors.

RESULTS AND DISCUSSION:
When supplemented into either a cost-effective DMEM/F12 basal media or commercially available CD medium Sheff-CHO CD system demonstrated improved overall CHO cell culture performance in terms of productivity when compared to unsupplemented media.

REF-B023

ANALYSIS OF THE PERFORMANCE OF SHEFFIELD RINSULIN IN MRC-5, MDCK AND CHO DG-44 CELL LINES

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KEY WORDS:
RECOMBINANT INSULIN / SERUM FREE MEDIA / GROWTH AND PROLIFERATION / SOMATIC CELLS / CHO CELLS

BACKGROUND AND NOVELTY:
In this study, the effect of 8 commercially available recombinant insulin's (rInsulin) including Sheffield's rInsulin ACF was studied on three industrially relevant cell lines MRC-5, MDCK. The cells were examined in normal culture conditions. The effect of all eight rInsulin on the same cells was also examined in serum depletion study, where the FBS was lowered to 5% for MRC-5 cells and 2% for MDCK. All the cell lines were treated with 10 µg/ml of each rInsulin. The cells were passaged for three successive subcultures and population doubling level was calculated. The results were compared against the same cells in medium untreated with insulin.

EXPERIMENTAL APPROACH:
The effect of Sheffield rInsulin ACF and the two best performing commercially available rInsulin was also investigated in CHO-DG44. A commercially available CD media was supplemented with 10mg/ml of each insulin, as was a media control containing no insulin. A shake flask experiment was performed for 14 days in triplicate. The cells were analyzed for effects on cell density and IgG titer over this period. The PDL of the MRC-5 and MDCK cells in normal conditions without serum was 47 hr, and 24 hr respectively. The PDL of the MRC-5 and MDCK cells in serum depleted conditions was longer at 59 hr and 30 hr respectively. The addition of all 8 insulin's led to a decrease in PDL in both normal and serum depleted conditions, with one exception. The average PDL in the rInsulin treated cell lines with serum depleted media dropped to a mean of 50 hrs in MRC-5 (max = 57 hr, min = 42 hr) and 23 hr in MDCK (max = 27 hr, min= 21hr). In all cases the biggest decrease in PDL was achieved with Sheffield's rInsulin ACF.

RESULTS AND DISCUSSION:
In the CHO DG-44 cell line it was observed that Sheffield rInsulin ACF was equivalent to the competitor products with little difference in cell growth or IgG titer observed. The media-only control showed poor cell growth and a reduction in IgG titer when compared to those supplemented with insulin.

REF-B024

METABOLIC FINGERPRINTING OF INSECT CELL LINES: DISCLOSING CELL LINE DETERMINANTS BEHIND SYSTEM'S PRODUCTIVITY

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KEY WORDS:
INSECT CELLS / BACULOVIRUS / METABOLOMICS / METABOLI PATHWAY ANALYSIS / VIRUS-HOST INTERACTIONS

BACKGROUND AND NOVELTY:
The Insect Cell-Baculovirus Expression Vector System (IC-BEVS) is a major biotechnological tool for the production of complex biopharmaceuticals. An in-depth metabolomic study concerning insect cells physiology and the subsequent impact of baculovirus will highlight major alterations caused by infection, but also key cellular features underpinning recombinant protein production. Moreover, it will provide clues to design better bioprocess optimization strategies.

EXPERIMENTAL APPROACH:
Two insect cell lines, Sf9 and Hi5, and two recombinant proteins, GFP and Neuraminidase, were compared. The respective exo- and endo-metabolome were assessed before and after infection with baculovirus for recombinant protein production. Exo-metabolome analysis comprised the quantification of sugars, amino acids and by-products. For the endo-metabolome, a total of 63 intracellular metabolites were identified and quantified by LC-MS.

RESULTS AND DISCUSSION:
Concerning recombinant protein production, Hi5 cells outperformed, with productivities 3 to 4 times higher than Sf9 cells. Multivariate analysis of intracellular metabolite profiles revealed significant differences between the two cell lines. Metabolites that contributed most to such differences included redox cofactors, osmolytes, and intermediates of urea cycle and pyrimidine metabolism. Metabolic pathway analysis showed that, regarding Sf9 cell line, infection strongly depends on the activation of amino acid metabolic routes, either for degradation towards feeding other pathways, or biosynthesis as product building blocks. For Hi5 cells, infection success relates to the activation of amino acids routes, TCA cycle, glycolysis, glutathione and nicotinamide metabolism, and lipid metabolism. Altogether, the results show that different metabolic landscapes are linked to distinct productivity phenotypes. The identification of metabolic pathways involved in the infection process and recombinant protein production will enable us to exploit new targets for further system's optimization.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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ASSESSMENT OF METABOLIC ALTERATIONS INDUCED BY ADENOVIRUS INFECTION IN HUMAN PRODUCER CELL LINES

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KEY WORDS:
ADENOVIRUS PRODUCTION / 1H-NMR / EXOMETABOLOME / METABOLIC FLUX ANALYSIS

BACKGROUND AND NOVELTY:
Adenoviruses constitute powerful vehicles for gene transfer with applications in vaccination and gene therapy. Several works have been done in optimizing the production of human adenovirus type 5 (HAV-5) in 293 cells, including feeding strategies to overcome depletion of nutrients and increase of toxic by-products. Meanwhile, alternative infection systems have been developed to overcome existing challenges. For instance, we have evaluated the capacity of a new human amniocytes derived cell line (1G3 cells) to produce HAV 5 which showed to be a good alternative to 293 cells. Developments in metabolic profiling have been providing knowledge on the mechanisms through which viruses manipulate the metabolic network of the host cell to supply energy and macromolecule components needed for replication and assembly. However, relevant data linking adenovirus infection and the metabolism of the host cell line is still lacking.

EXPERIMENTAL APPROACH:
The main focus of this work was studying the metabolic impact of HAV-5 infection in two producer cell lines (293 and 1G3), and try to gain insights into the cell density effect which occurs at higher densities for 1G3 cells. The exometabolome of both cell lines, infected at different concentrations in shake flask and bioreactor cultures, was analysed by 1H-NMR spectroscopy. The early and late times of infection will be analyzed by metabolic flux analysis to evaluate how the virus manipulation of the cell metabolism evolves in both cell lines.

RESULTS AND DISCUSSION:
The use of 1H-NMR allowed a comprehensive metabolic characterization of cell supernatant during growth and infection phases, including quantification of metabolites which are not routinely measured in mammalian cells. These data will be contextualized into a metabolic network and the main differences between the metabolism of the two producer cell lines at different stages of infection, will be highlighted. These results will allow proposing strategies to optimize adenovirus production at higher cell densities.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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INSECT CELL EXPRESSION OF COMPLEX PROTEINS THROUGH STABLE TARGETED INTEGRATION

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KEY WORDS:
PRODUCTION OF COMPLEX PROTEINS / RMCE-SF9 DERIVED CELL LINE / NEURAMINIDASE PROTEIN / ROTAVIRUS VP2 PROTEIN

BACKGROUND AND NOVELTY:
The insect cell/baculovirus expression system (BEVS) has been widely exploited to produce complex proteins, as it allows high protein expression levels in relatively short development timelines. However, this expression system has some limitations associated, namely i) its lytic nature, affecting the efficiency of the cellular protein processing machinery in late stages of infection (proteins requiring complex processing are often produced with low quality), ii) the added burden to maintain the viral stock and iii) the genetic instability of baculovirus vectors. As an alternative, a flexible Sf9 insect cell line was recently developed leveraging the recombinase-mediated cassette exchange (RMCE) approach [1], which resembles the “plug and play” concept of the baculovirus system with similar short development timelines and overcoming the limitations associated with the transient nature of BEVS. In this work, we will evaluate the capacity of this cell line to express two markedly different proteins: the soluble form of influenza virus neuraminidase (NA1) glycoprotein and the rotavirus inner capsid protein VP2, which self¬-assembles into rotavirus core-like particles.

EXPERIMENTAL APPROACH:
The genes coding for NA1 and VP2 proteins were integrated into a pre-tagged locus of the RMCE-Sf9 cell line through flipase-mediated recombination. The performance of each resulting cell line was evaluated and compared with that obtained from baculovirus infection. Finally, cell culture optimization was performed based on metabolic characterization of the producer cell lines.

RESULTS AND DISCUSSION:
Although baculovirus infection displays higher specific productivities, stable expression can provide better volumetric protein titers through rational feeding strategies, allowing high cell densities (20x106 cells/ml) and demonstrating the potential of the RMCE-Sf9 cell platform.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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OTHER INFORMATION
FF, JV and MD contributed equally to this work

DEVELOPING A SIMPLE ROBUST AMINO ACID SPECIFIC ISOTOPIC LABELING PROTOCOL IN INSECT CELLS USING BIIC BACULOVIRUS

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KEY WORDS:

ISOTOPIC LABELING / BACULOVIRUS / INSECT CELLS / DFG-IN/OUT / NMR

BACKGROUND AND NOVELTY:

Isotopic protein labeling and NMR are powerful tools in drug discovery for structure based drug design programs. Together these technologies can be applied to a wide variety of protein structural studies including: NMR fragment based screening and lead generation for HTS, determination of DFG –in/out motif for inhibitor design strategy, allosteric binding site determination and characterization, mechanistic studies of compound binding and ligand interactions, pKa determination and even SeMet labeling for phase determination for x-ray crystal structure.

EXPERIMENTAL APPROACH:

Baculovirus expression vector system (BEVS) is a frequently used method for the production of soluble recombinant protein expression in structural biology programs due to their ability to perform posttranslational modifications. However specific amino acid labeling in insect cell baculovirus systems present significant challenges as literature labeling strategies offer significant method variation. Insect labeling media are not only complex, but they can be expensive. A reproducible infection method must be empirically established with liquid virus stocks which by nature are often unstable and suffer from batch variability between amplification and titering over time. Additionally, high levels of specific label substitution/incorporation, requires the absence of unlabeled amino acids from the media, or a depletion of any intracellular pools of the amino acid target, to minimize isotopic label dilution.

RESULTS AND DISCUSSION:

Here we present a simple, robust method for isotopic labeling of specific amino acids in insect cells using BIIC (Baculovirus Infected Insect Cells) baculovirus - a novel application of BIIC technology. The advantages of the new labeling process are many fold: (1) elimination of virus amplification and titering - enabling reproducible expression consistency and efficiency (2) simple, rapid method – a 96 hour total process time (3) process yields of >1mg/L purified protein with >90% label incorporation with 13C or 15N amino acid substitution confirmed by mass spectrometry (4) the labeling media cost is less than traditional labeling media. Applications of this novel isotopic labeling method, across the structure based drug discovery portfolio, (SPHK-1, ITK, BTK and Jak3) will be presented.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

Pfizer Worldwide Research and Development, Groton, CT Providing expression scale-up support of protein targets and reagents for Research Units and Structural Biology.

Primary responsibilities include: Support Research Unit discovery programs by providing expertise in the expression of recombinant proteins for HTS, assay development and structural biology. Primary expertise in expression optimization and technology development to express protein targets in mammalian, insect cell/baculovirus and bacterial cell lines: including hybridoma antibody, plasmid DNA preparation, transient transfection, BIIC (baculovirus infected insect cell) and BacMam transduction technology. Utilization of a variety of bioreactor technologies including Wave, BelloCell, Applikon and Bioflo-4 SIP benchtop fermentors, NBS IF-150 microbial fermentor and NBS 6000 mammalian cell bioreactors. Protein purification and characterization in affinity protein purification systems, technology development in selenomethionine labeled CHO expression systems, 15N and 13C-labeling of E. coli expressed proteins and isotopic labeling of specific amino acids in baculovirus insect cell culture.

STUDY OF PERFUSION PROCESS OF CHO CELLS IN BENCH-TOP SINGLE-USE-BIOREACTORS (SUB) WITH 150 ML MATRIX

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KEY WORDS:
SINGLE-USE-BIOREACTOR / PERFUSION CULTIVATION / MAMMALIAN CELL LINE / ANTI-BODY EXPRESSION / ON-LINE BIOMASS MONITORING

BACKGROUND AND NOVELTY:
For reduced facility and equipment investment a perfusion Single-Use-Bioreactor (SUB) for efficient production of recombinant products from mammalian cell lines was developed from rigid plastics. In the CellTank SUB both adherent and suspension mammalian cell lines are harbored in the novel CellCore environment composed of stacked OD 80 mm matrix discs caged each in parallel arranged envelopes with 1 liter re-circulating media and pump all immersed in the reservoir. CellCore are scalable by altering the diameter and the number of matrix discs generating a working volume spanning >1:1,000 from few mL to 15,000 mL.

EXPERIMENTAL APPROACH:
In this study, suspension-adapted Chinese Hamster Ovary (CHO) cells producing IgG monoclonal antibody was inoculated at low density and cultivated in the CellTank bench-top SUB containing 150 mL polyester CellCore fiber matrix. A real-time biomass sensor using the dielectric properties of living cells was used to measure the live cell density. The operation and performance of the setting was studied 15 days in batch followed by 11 days in perfusion cultivation with 19,500 mio cells.

RESULTS AND DISCUSSION:
A viable cell density of 200 mio cells/mL was achieved at perfusion rate 10 RV/day (Reactor Volume) during a first run. With the biomass sensor it was found that 1 pF/cm was equivalent to 1 mio viable cells/mL. In a second run, viable constant cell density at 130 pF/cm was maintained over 11 days at 8 RV/day perfusion rate at reduced temperature. The cell specific productivity was comparable or higher and the volumetric IgG productivity was up to 61 times higher than cultures in Erlenmeyer flasks. No retention of IgG was found in the CellCore matrix. As a bench-top SUB, the CellTank system integrating the cell retention matrix can work long-term and stable at cell density above 100 mio viable cells/mL without IgG retention. Found to be easy and handy to operate, CellTank offers a solution alleviating technical and sterility challenges occurring in traditional perfusion processes.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Dr. Veronique Chotteau is Principal Investigator, managing the Cell Technology Group at KTH since 2008. She was recruited to KTH from the biopharmaceutical industry Pharmacia Upjohn / Biovitrum due to her industrial experience in mammalian cell processes from small to commercial GMP scale, in project management of biopharmaceutical process development (e.g. recombinant factor VIII Refacto©, monoclonal antibodies) and in business development. Dr. Chotteau has 25 years of experience in mammalian cell cultivation and 12 years of experience in biopharmaceutical industry, including international collaboration with industrial partners in USA and Europe, ranging from BigPharma to SME.

OTHER INFORMATION
On-line measurement with long-time stable Single-Use-Sensor´s for pH, DO, glucose and lactate has recently been developed in collaboration with Hamilton and C-CIT and added to the CellTank SUB.

REF-B029

MODELING OF AMINO ACID METABOLISM IN CHO CELL CULTURE: A ONE-MODEL APPROACH FOR MULTIPLE METABOLIC STATES USING EXTRACELLULAR MEASUREMENTS

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KEY WORDS:
CHO CELLS / MODELING / ELEMENTARY FLUX MODES / METABOLIC FLUX ANALYSIS / AMINO ACIDS

BACKGROUND AND NOVELTY:
Metabolic flux modeling can provide deeper understanding of metabolic states triggered under different cultivation conditions, as well as potential for simulation and optimization. The complexity of mammalian cell metabolism makes model construction a challenging task. Elementary flux modes (EFMs) can be used to form macroscopic models in which each EFM represents one path through the network [1,2]. This links extracellular components and alleviates the need for intracellular metabolite measurements. Multiple metabolic states, e.g. those obtained in batch mode culture, have been described by sets of EFM models, each model describing a cultivation phase or condition [2]. Our aim was to develop a method to create one single model, able to predict the behavior of a cell culture under varying environmental conditions and using extracellular component measurements only. We propose a methodology based on EFM analysis and use it to generate a model from data obtained in Chinese Hamster Ovary (CHO) cell cultures.

EXPERIMENTAL APPROACH:
A metabolic network representing important metabolic pathways was constructed. EFMs were computed using the Metatool algorithm [3] and internal metabolites eliminated by algebraic operation. The flux in each EFM, i.e. in each feasible path through the network, was modeled by Michaelis-Menten kinetics. Chemically defined media differing in the composition of amino acids were used to cultivate an antibody producing CHO cell line in pseudo-perfusion mode with daily medium renewal and sample collection for analysis of cell number and important extracellular metabolites.

RESULTS AND DISCUSSION:
Varying amino acid concentrations resulted in a data set representing multiple metabolic states. The whole set was used for model fitting to create one single model by generating maximal kinetic rate constants using non-negative least-squares optimization that minimized the difference between experimental data and the model-derived rates. A good fit between model and experimental data was observed.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-B030

SAMPLING AND QUENCHING OF CHO SUSPENSION CELLS FOR ANALYSIS OF INTRACELLULAR METABOLITES

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KEY WORDS:
QUENCHING / CHO / INTRACELLULAR METABOLITES / METABOLOME ANALYSIS / METABOLISM

BACKGROUND AND NOVELTY:
Metabolic studies are of fundamental importance in metabolic engineering approaches to understand cell physiology and to pinpoint metabolic targets for process optimization. Knowledge of intracellular metabolites is an essential requirement to obtain a comprehensive understanding of metabolism, e.g. about metabolic compartmentation. Few protocols for quantitative analysis of intracellular metabolites in mammalian suspension cells have been proposed in the literature. However, due to limited validation of sampling and quenching procedures provided in previous publications, we thoroughly investigated the associated critical issues. In addition, we highlight pit-falls and potential sources of error.

EXPERIMENTAL APPROACH:
We developed a simple sampling and quenching protocol for mammalian suspension cells using 0.9% saline for quenching and washing as previously proposed (1). Cells were harvested by centrifugation. Critical issues, such as (a) cellular integrity, (b) quenching efficiency, (c) cell separation at different centrifugation conditions and its influence on cell fitness, and (d) different washing procedures to prevent carryover of extracellular metabolites, have been investigated.

RESULTS AND DISCUSSION:
Ice-cold 0.9% saline was shown to be a suitable quenching solution (QS) maintaining cellular integrity as reported previously (1). A rapid temperature shift was achieved using a nine-fold excess of QS resulting in inactivation of metabolism as shown by a high energy charge value. The applied conditions result in a very low level of medium contamination. Rinsing the cell pellet without re-suspending the cells prevented medium carryover effectively. Separation of cells via centrifugation was incomplete due to required short centrifugal times. Thus, it is necessary to determine the cell recovery after quenching.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-B031

INVESTIGATION OF GLUTAMINE METABOLISM IN CHO CELLS BY DYNAMIC METABOLIC FLUX ANALYSIS

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KEY WORDS:
CHO CELLS / GLUTAMINE METABOLISM / METABOLIC FLUX ANALYSIS / FLUX BALANCING ANALYSIS

BACKGROUND AND NOVELTY:
The glutamine metabolism represents one of the major targets in metabolic engineering and process optimization approaches due to its importance as cellular energy, carbon and nitrogen source. We used Dynamic Metabolic Flux Analysis to investigate the glutamine metabolism in CHO K1 cells capturing the metabolic dynamics during batch- and fed-batch cultivations.

EXPERIMENTAL APPROACH:
We tested different glutamine start concentrations and different glutamine feeding profiles to study the impact of glutamine availability or limitation on the physiology of CHO K1 cells. Dynamic Metabolic Flux Analysis was applied for a time-resolved description of growth and metabolism in batch and fed-batch cultivations.

RESULTS AND DISCUSSION:
The different glutamine batch- and fed-batch conditions had no significant impact on glycolysis and lactate excretion. However, substantial flux rearrangements were observed for TCA cycle and amino acid metabolism. Pyruvate consumption was increased with decreasing glutamine start concentration. At glutamine free conditions, pyruvate was converted into oxaloacetate to fuel the TCA cycle. Serine uptake and biosynthesis as well as glycine and C1-unit formation were increased at low glutamine start concentrations and highest under glutamine free conditions. Excretion of glutamate was highest in the glutamine free cultivation and lowest at low glutamine concentrations. TCA cycle fluxes were significantly increased with increasing glutamine concentrations and anaplerotic fluxes were reversed to form pyruvate from oxaloacetate. However, secretion of alanine and ammonia was also increased. In the late cultivation phase, overall higher fluxes were maintained for the low glutamine and glutamine free conditions compared to higher glutamine start concentrations. The fed-batch cultivations showed an intermediate behavior. Waste product formation was reduced compared to batch cultivation. TCA cycle fluxes showed less variation during the cultivation process and were longer maintained at higher values.

REF-B032

13C-LABELING DYNAMICS OF INTRA- AND EXTRACELLULAR METABOLITES IN CHO SUSPENSION CELLS

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KEY WORDS:
CHO CELLS / ISOTOPE LABELING / INTRACELLULAR METABOLITES / METABOLISM / 13C-NON STATIONARY METABOLIC FLUX ANALYSIS

BACKGROUND AND NOVELTY:
Isotope labeling techniques have become a most valuable tool in metabolomics and fluxomics. In particular the dynamics of label incorporation provide rich information about the metabolism. A thorough understanding of CHO metabolism is crucial for metabolic engineering and process optimization approaches.

EXPERIMENTAL APPROACH:
A non-stationary 13C-labeling experiment with [U-13C6]glucose and [U-13C5]glutamine was performed in CHO suspension cells. Growth, extracellular metabolite concentrations as well as labeling dynamics of extra- and intracellular metabolites were analyzed.

RESULTS AND DISCUSSION:
The two tracers, glucose and glutamine, were found to be the major carbon sources. We observed net excretion of lactate, alanine, glycine and glutamate and net consumption for pyruvate and all other amino acids. Metabolic steady state was confirmed by exponential growth and constant yields (1). Although only net consumption of serine, glutamine and aspartate was observed, a significant part of these metabolites was found labeled indicating parallel synthesis and consumption. Using glucose as tracer label incorporation into lactate was very fast reaching intra- and extracellular isotopic steady state. Label incorporation into alanine was slower and the labeling pattern of intracellular alanine was different to that of pyruvate, lactate and extracellular alanine. Labeling in lactate and alanine was also found using glutamine as tracer indicating a reflux from TCA cycle via anaplerotic reactions. Label incorporation into TCA cycle was fast from both tracers reaching isotopic steady state in citrate within the first 6 h of cultivation. Nearly identical labeling patterns were found for fumarate, malate and aspartate using both tracers indicating a very tight connection between these pools.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-B033

FUNCTIONAL NON-VIRAL MIRNA DELIVERY INTO PHARMACEUTICAL PRODUCTION CELL LINES: BREAKING LIMITATIONS OF COMPLEX CULTURE MEDIA

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KEY WORDS:
MICRORNA / CHO / CAP / TRANSFECTION

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) play a central role in many cellular processes as key regulators of gene expression. They function by translational repression or mRNA decay and have recently been described as promising targets for cell line engineering strategies. Careful examinations of miRNA function and their specific gene targets in pharmaceutical production cell lines call for an efficient delivery of functional miRNAs into these cells. However, non-viral nucleic acid transfer mediated by cationic lipids or polymers is often hampered by complex media. The aim of the study was to identify a scalable method for efficient miRNA delivery into cells grown in complex culture media which would enable to effectively modify cellular phenotypes.

EXPERIMENTAL APPROACH:
MicroRNA-mediated gene regulation can be facilitated by directly introducing miRNA duplex intermediates (miRNA mimics) into cells. Argonaute proteins in the cytoplasm then capture these exogenous miRNA mimics, resulting in the regulation of specific target genes. Different cationic lipids and polymers were tested for their ability to transfect Chinese hamster ovary (CHO) and Cevec's Amniocyte Production (CAP) cells, grown in rich media. Fluorescently labeled RNA duplexes were used to measure transfection efficiency by flow cytometry and fluorescence microscopy. In addition, confirmation of functionality of transferred miRNAs was shown by Twf-1 knockdown following miR-1 delivery. Finally, CHO and CAP cells were transfected with species-specific death target siRNAs and cellular analysis was performed by quantitative flow cytometry.

RESULTS AND DISCUSSION:
We identified a highly suitable transfection reagent exhibiting exceptional miRNA and siRNA transfection efficiency. Furthermore, the reagent was unique in its ability to induce remarkable changes on mRNA, protein as well as phenotypic levels in CHO and CAP cells cultivated in rich media. The provided transfection method should promote efforts to discover novel target miRNAs for cell line development in the future.

REF-B034

IDENTIFICATION OF PROCESS RELEVANT MIRNA IN CHO CELLS

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KEY WORDS:
MIRNA / CELL LINE ENGINEERING / CHO CELLS / BIOPROCESS

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are small RNAs which function as regulators of posttranslational gene expression by binding to their mRNA targets. MiRNAs are involved in crucial regulations of many signaling and metabolic pathways. In difference to other interfering RNAs (RNAi), miRNAs can target many mRNA, thus having an increased impact on regulation of gene expression. These properties of miRNAs makes them interesting and promising targets for biomarkers and cell line engineering. Therefore, we studied miRNA profiles during different culture phases and process conditions and investigated the potential of differentially expressed miRNAs as targets for process optimization. This may help to pave the way to introduce a new layer of control for cell line engineering.

EXPERIMENTAL APPROACH:
In this study miRNA profiles of Chinese hamster ovary (CHO) cells during different Batch culture phases with and without temperature shift were established. CHO-DG44 cells were cultivated in 2L bioreactors. For the control runs temperature was maintained at 37°C all time, while for the temperature shift the temperature was reduced to 30°C. MiRNA profiles for five different time points and culture phases for each condition were studied in cross species microarray chips. Results were confirmed with RT-PCR. To identify CHO specific miRNAs, deep sequencing Illumina technology was applied.

RESULTS AND DISCUSSION:
The different culture stages in the control runs, during the temperature shift and when these two process conditions are compared is clearly visible on the miRNA profiles. MiRNA expression pattern changes when cells enter different culture phases. Temperature shift has a high impact on miRNA profiles. With the high time resolution of our approach we can discriminate between early and late responses to the temperature shift. Directly after the temperature shift many miRNAs are up regulated. These miRNAs return to normal levels after a short time, indicating that miRNA are one of the first responses when cells adapt to a new environment.

FULL TRANSCRIPTOME ANALYSIS OF CHINESE HAMSTER OVARY CELL LINES PRODUCING A DYNAMIC RANGE OF COAGULATION FACTOR VIII

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KEY WORDS:
NEXT GENERATION SEQUENCING / HETEROLOGOUS PROTEIN PRODUCTION / COAGULATION FACTOR VIII / CHINESE HAMSTER OVARY CELL LINES

BACKGROUND AND NOVELTY:
Coagulation Factor VIII (FVIII) is an essential cofactor in the blood coagulation cascade. Inability to produce functional FVIII results in haemophilia A which can be treated with recombinant FVIII. Chinese Hamster Ovary (CHO) cells are the most used cell line for producing complex biopharmaceuticals due to its ability to perform complex post-translational modifications. When mammalian cells overexpress a protein like FVIII they will adapt by regulating various proteins and pathways to support synthesis/production of this protein. Yields of FVIII produced in CHO are low and for this reason a greater understanding of what constitute a high producing cell line is desired. In this study a full transcriptome analysis was undertaken in order to analyze the differences between high and low producers of FVIII.

EXPERIMENTAL APPROACH:
The FVIII gene was introduced into CHO-DUKX-B11 cells and a stable pool was generated by selection with MTX. A number of subclones were analysed and 3 high producing clones, 3 medium producers and 3 low (~ 0) producer clones were isolated. These 9 clones were grown in shake flasks in batch culture. During the cultivation essential metabolites were monitored as well as cell number and viability. RNA was extracted after 48 hours of fermentation and sequenced using the Illumina HiSeq system. Reads were processed and aligned to the CHO-K1 genome using Tophat and expression levels were deduced using DESeq.

RESULTS AND DISCUSSION:
Experiments showed that 48 hours into the cultivation cells were seen to grow in the exponential phase in media still containing sufficiently high amounts of glutamine and low amounts of lactate. Furthermore, a significant difference in FVIII levels was detected at this time in the media of cells from the different groups and for this reason this time point was chosen for extraction of RNA. The results from the total analysis of RNA-seq data will be presented and tendencies found to correlate with productivity of coagulation factor VIII will be investigated and discussed.

A VALIDATED SYSTEM FOR LIGATION-FREE USER™ -BASED ASSEMBLY OF EXPRESSION VECTORS FOR MAMMALIAN CELL ENGINEERING

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KEY WORDS:
MAMMALIAN EXPRESSION VECTORS / LIGATION-FREE CLONING / URACIL-EXCISION / HIGH-THROUGHPUT CLONING / MULTIPLE PCR FRAGMENT ASSEMBLY

BACKGROUND AND NOVELTY:
The development of mammalian cell factories requests for fast and high-throughput methods, which means a high need for simpler and more efficient cloning techniques. For allowing fast and flexible vector construction for optimization of protein expression by genetic engineering and metabolic engineering in mammalian cells, a new versatile expression vector system was developed. This vector system applies the ligation-free USER™ (uracil-specific excision reagent) cloning technique to rapidly construct mammalian expression vectors of multiple parts and with maximum flexibility.

EXPERIMENTAL APPROACH:
Vector parts are PCR-amplified separately and can be combined as preferred in a very fast and easy manner. This allows fast and dynamic gene, promoter or selection marker swaps. In particular, the uracil excision cloning facilitates easy preparation of fusion proteins. Thus, fluorescence, purification or localization tags can easily be integrated. All vectors assemblies were verified by sequencing. The functionality of the vectors was tested for transient expression of fluorescent model proteins in CHO, U-2-OS cells and HEK293 cell lines.

RESULTS AND DISCUSSION:
A novel USER cassette free assembly system was created based solely on PCRs product for assembly of multiple parts. The vector system was tested with many of the most common vector elements for heterologous gene expression in mammalian cells. Furthermore, additional parts were included for subcellular locations with defined N- and C-terminal linkers verified for functionality as well as parts for bicistronic expression. The created system allowed assembly of at least 7 parts simultaneously with correct directionality and with a cloning efficiency above 93%. The functionality of the assembly system and the standardised parts were validated by detection of fluorescent expression from a series of constructed vectors expressed in different mammalian cell lines. To our knowledge no other comparable assembly system for mammalian expression vectors exists.

REF-B037

STUDY OF RELATIONSHIP BETWEEN OXIDATIVE METABOLISM AND ANTIBODY SPECIFIC PRODUCTION RATE IN INSULIN-GLUCOSE SUPPLEMENTED CONTINUOUS CULTURES OF RECOMBINANT NS0 CELL LINE

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KEY WORDS:
SPECIFIC PRODUCTION RATE / GLUCOSE METABOLISM / NS0 CELL LINE / CONTINUOUS CULTURE / INSULIN

BACKGROUND AND NOVELTY:
Among mammalian cell lines, NS0 is one of the most used for monoclonal antibodies (mAb) production. Culture medium components optimization and characterization of cell metabolism had contributed to increase cell growth, reaching higher specific products concentration and gross productivity. In particular, strategies based on manipulation of signal pathways that influence cell growth and survival, energy-consumption and protein synthesis have been addressed. Continuous cell culture allows the establishment of well-defined steady states. It is determinant to evaluate the relationship between the concentration of substances inside the bioreactor and biological reaction rates. In particular, the modified GS-NS0 cells with Glutamine Synthetase (GS) expression technology, is universally used in the biotechnology industry. However, applications of NS0 cells without GS technology are still barely studied at industrial level. The goal of this work is to study the relationship between the oxidative metabolism and antibody specific production rate in insulin-glucose supplemented continuous cultures of recombinant NS0 cell line with no expression of GS.

EXPERIMENTAL APPROACH:
The NS0 cell line expresses an IgG1 recombinant humanized monoclonal antibody. Cells were cultured in stirred tank bioreactors. The basal medium MyeloCIM (developed in-house) is constituted by equal proportions of RPMI:DMEM-F12:IMDM supplemented with amino acids, vitamins, iron salt and a phospholipid. Added glucose concentrations: 17, 25, 35, 45 and 50 mmol /L, with or without insulin. Levels of G6PDH, mTOR, Akt, eIF2 and GLUT1, GLUT4 were detected by FACS and western blotting. Enzymatic activities of G6PDH, LDH, PYK, glutaminase and ALAT were measured. Glucose and lactate concentrations were determined by using an YSI analyzer. The extracellular IgG was assayed by sandwich ELISA. Intracellular IgG and apoptosis were detected by FACS. ADP/ATP ratio was measured by bioluminescence assay.

RESULTS AND DISCUSSION:
In glucose and insulin supplemented medium, NS0 cells increase twice specific mAb production rate. An enhancement in glucose transporters, mTOR, Akt and eIF2 expression, as well as increase activity of G6PDH, PYK and ALAT is detected. The addition of insulin induces a 2-fold increase in glucose uptake; meanwhile the residual lactate remains constant with observed reduction in LDH activity. The increases in glucose concentration in the culture medium as well as insulin addition reduce the fraction of apoptotic cells. Obtained results indicate that insulin and glucose addition to growth medium of NS0 cell line orchestrate modifications in energetic metabolism and cells capacity to produce recombinant mAbs by concerted action of cell survival, protein biosynthesis and glucose metabolism associated mechanism.

REF-B038

INVESTIGATION OF THE EFFECT OF PH ON CHO CELL GROWTH AND IGG4 PRODUCTION AT DIFFERENT SCALES

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KEY WORDS:
CHO CELLS / MICROPLATES / PH / STR BIOREACTOR

BACKGROUND AND NOVELTY:
The production of recombinant therapeutic proteins in mammalian cells plays a big part in the biopharmaceutical industry. Consequently, improvements to the process have to be performed. One key parameter that has a big impact in the cell growth, productivity, cell metabolism, protein glycosylation is the pH. With the link of novel engineering approaches with knowledge of the cell culture and physiology is possible to establish an automated microwell approach to mammalian cell culture that simulates the dynamic and heterogeneous environment found in large-scale culture vessels.

EXPERIMENTAL APPROACH:
An industrial GS-CHO cell line producing a whole IgG 4 was cultured in 24-Standard Round Well (24-SRW) microtitre plates at different incubator's carbon dioxide atmospheres. Non-invasive measurements of pH in 24-SRW microplates cultures were made using a SensorDish Reader coupled with a 24-SRW HydroDish from PreSens, Germany. Stirred tank reactor (STR) experiments were performed using a 5-L BIOSAT B-DCU with a working volume of 3.5 L at 37C, 30% dissolved oxygen air saturated and at different pH.

RESULTS AND DISCUSSION:
In both scales, 24-SRW microplate and 5L STR, IgG4 productivity is the highest at acidic condition, and it is similar for neutral and basic conditions, however cell production is higher under basic conditions. Other phenomena observed was the long batch time and larger cells in pH 6.8 STR, where the maximum viable cell concentration reached is 3.5x106 cells mL-1 with no lactate production. The lactate and ammonia formation is larger at smaller scale, probably due to lack of pH control. It is also observed lactate consumption when glucose levels reached below 2 g L-1. At 24-SRW microplates a carbon dioxide atmophere higher than 10% (V/V) is proved to be toxic to the cell growth and the cell size distribution during culture time was similar between different carbon dioxide atmosphere. It was possible to use microplates as a pH scale-down model of pilot plant bioreactors to predict the IgG4 productivity.

ENGINEERING THE ENERGY METABOLISM AND LACTATE PRODUCTION IN MAMMALIAN CELLS: DOWN-REGULATION OF THE WARBURG EFFECT

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KEY WORDS:
WARBURG EFFECT / HIF1 (HYPOXIA INDUCIBLE FACTOR 1) / PDK (PYRUVATE DEHYDROGENASE KINASE) / LACTATE / ENERGY METABOLISM

BACKGROUND AND NOVELTY:
Most mammalian cell lines used in biopharmaceuticals production accumulate significant amounts of lactate – derived by the Warburg Effect (WE) – decreasing final product titers and quality as well as reducing culture viability. Engineering approaches to revert this phenotype have mainly targeted the lactate dehydrogenase reaction, by reducing or by-passing it, translated in titer improvements up to 3-fold. We conducted a proof-of-concept metabolic engineering study based on the down-regulation of two key molecular effectors of the WE, hypoxia inducible factor 1 (HIF1) and pyruvate dehydrogenase kinase 1 and 3 (PDK1/3), achieving titer improvements of more than one order of magnitude.

EXPERIMENTAL APPROACH:
Down-regulation was performed in HEK 293 cells producing recombinant retrovirus using lentiviral vector mediated short-hairpin RNA interference.

RESULTS AND DISCUSSION:
For the best HIF1 silenced clone an 18-fold increase in total particles (T.P.) and a 22-fold increase in infectious particles (I.P.) production was achieved. LDH activity was decreased by 60% and glucose uptake/ lactate production rates were reduced by more than 2-fold. Further silencing of PDK potentiated the effects of HIF1 down-regulation and resulted in additional improvements both in viral titers and in viral preparation quality. Interestingly, the best synergistic effects on the accumulation of PDK reduction did not occur in the highest-titer HIF1 down-regulated clone: the expression level of these two genes requires fine tuning for maximum productivity increases. Thus, the physiological fingerprints associated to these manipulations are under analysis including the metabolic and enzyme activity profiling of central carbon metabolism as well as a microarray based transcriptome analysis. These results highlight the manipulation of the Warburg Effect as a potential metabolic engineering tool for improving the production of complex biopharmaceuticals in cultured mammalian cell lines.

A VALIDATED SYSTEM FOR LIGATION-FREE USER™ -BASED ASSEMBLY OF EXPRESSION VECTORS FOR MAMMALIAN CELL ENGINEERING

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KEY WORDS:
ENZYME PROTEIN THERAPEUTIC / CHO CELL CULTURE / METABOLIC SHIFT

BACKGROUND AND NOVELTY:
Production of recombinant lysosomal enzymes using Mannose-6-Phosphate Receptor (M6PR) for lysosomal targeting has a potential to interfere with the normal operation of the M6PR pathway in the production cells. We have demonstrated major changes in the lysosomal function in CHO cells caused by the saturation of the M6P receptors during production of such recombinant enzymes.

EXPERIMENTAL APPROACH:
We will describe examples to this phenomenon and potential cell culture strategies to mitigate the undesirable effects.

RESULTS AND DISCUSSION:
As a result of altering the lysosomal functions, unusual metabolites are released by the host cell into the medium. These may include glycosaminoglycans, lysosomal enzymes, etc. that are not detectable (or are only present in significantly lower quantities) in the culture supernatants of the untransformed host cells.

REF-B041

CELL LINE SCREENIG FOR ANTIBODY-PRODUCING CELLS USING SPLIT GREEN FLUORESCENT PROTEIN

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KEY WORDS:
SPLIT GFP / CELL LINE DEVELOPMENT / ANTIBODY / CHO

BACKGROUND AND NOVELTY:
The establishment of high antibody-producing cell line is an important issue in biologics production. An efficient high-throughput cell screening technology needs to be developed from a large number of candidate cells. For the indirect detection of a target protein, intracellular fluorescent proteins such as green, red and yellow fluorescence proteins have widely used because of easy activation without cofactors and substrates. Additionally fluorescence-activated cell sorting-based cell line development is an efficient strategy with the benefits of flow cytometry. Two color fluorescent proteins-based flow cytometry method was already reported to select clones possessing a high concentration of the heavy and light chain of antibody, but limited in terms of delicate relationship between two-fluorescence intensity and antibody production.

EXPERIMENTAL APPROACH:
The heavy and light chain genes of fully human anti-angiogenic monoclonal antibody were kindly provided from Pharmabcine Corporation in Korea. An internal ribosome entry site was introduced for using green fluorescence protein (GFP) fragments (N-terminal and C-terminal fragments) as a reporter to the heavy and light chains of antibodies. The CHO-K1 cells were used and cultivated in RPMI media with 10% fetal bovine serum. FACSAria system was used to estimate the green fluorescence intensity of various clones.

RESULTS AND DISCUSSION:
The FACS-sorted cell pool containing reconstituted GFP had higher antibody productivity than the unsorted cell pool. The fragment complementation of split GFP was an efficient subsidiary indication for antibody production. As a result, the antibody titer was highly correlated with the fluorescence intensity, demonstrated from the analysis of median GFP and specific antibody productivity in individually selected clones. FACS-based cell screening method is expected to be a powerful tool for high antibody-producing cell line development.

REF-B042

ENDOGENOUS CHO PROMOTERS AS BIO-ENGINEERING TOOLS

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KEY WORDS:
CHO CELLS / ENDOGENOUS / PROMOTER / BIOPHARMA / TEMPERATURE SHIFT

BACKGROUND AND NOVELTY:
I aim to identify and exploit temperature sensitive CHO promoters (via genomic studies) in order to create tools to control expression and improve biopharmaceutical protein production. By using inducible endogenous CHO promoters I hope to fill a niche where the uses of these promoter switches can be used in conjunction with other areas of the project and add to existing tools used currently. Although novel inducible promoters are the main focus I have also concentrated on obtaining potentially strong novel constitutive promoters from CHO genes that show strong expression from our microarray and TLDA analysis which can also hold benefits in that if you have a stable cell line with strong endogenous promoter versus say a commercial viral one, chances are that over time stability will prevail stronger for longer and not succumb to mutations etc and silencing issues as seen in heterogeneous systems.

EXPERIMENTAL APPROACH:
Cross-species alignments to assess comparisons between CHO and Mouse and Rat homologs for example, PCR and RT-qPCR methods to extract the said promoter and gene fragments (note: i began before the CHO sequence became available), Restriction digestion of promoter regions and cloning into GFP, Luciferase and EPO expression vectors for Functional Validation and testing of isolated novel promoters. Bioinformatic analysis on the promoters of interest to understand important sections and essential sequence information which makes the promoter work, mutational anaylsis to illustrate the temperature sensitive regions and strength.

RESULTS AND DISCUSSION:
Currently I have ~30 CHO sequences of varying length from 9 different genes, and after functional validation I have strong reason to believe 3/4 of these isolated sequences have great potential to control and drive expression in a variety of systems and can be used to drive difficult proteins. I have isolated 2 temperature sensitive promoters which show to be ~5 fold increase in expression at 31oC temp shift and 37oC temp shift respectively.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Xu et al, 2011 - The genomic sequence of the Chinese hamster ovary (CHO) -K1 cell line

OTHER INFORMATION
Concept of Viral promoters versus Endogenous promoters. Endogenous are obviously more appealing to large multinational biopharma companies due to FDA approval concerns and safety issues arrising from heterogenous methods, this i believe shows the novel aspect. Gene sets selected based on strong bioinformatics and microarray profiling data.

PROFILING AND ENGINEERING OF MICRORNAS FOR ENHANCING RECOMBINANT PROTEIN PRODUCTIVITY IN CHINESE HAMSTER OVARY CELLS

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KEY WORDS:
MICRORNA / CHINESE HAMSTER OVARY CELLS / STABLE OVER-EXPRESSION / RECOMBINANT PROTEIN PRODUCTION / SPECIFIC PRODUCTIVITY

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO) cells have become dominant host cells in the biopharmaceutical industry due to their capacity for proper protein folding, assembly and post-translational modifications. However, low specific productivity (qp) places limitations on yields obtained from mammalian host cells. MicroRNAs (miRNAs), a novel class of short, non-coding RNAs which negatively regulate target gene expression at post-transcriptional levels, have emerged as promising targets for engineering of CHO cell factories to enhance recombinant protein production. While engineering of miRNAs for enhanced cell growth and delayed cell death have been reported, miRNA targets which can enhance qp have not been identified to date.

EXPERIMENTAL APPROACH:
To understand the role of miRNAs in conferring high qp phenotype in CHO cells, we carried out high throughput sequencing of 4 in-house generated IgG-expressing CHO sub-clones of varying qps. Reads were mapped to miRBase and 22 miRNAs were found to be differentially expressed between the high and low producers. These miRNAs were stably transfected into an IgG-expressing sub-clone to assess their effects on growth, titer and qp.

RESULTS AND DISCUSSION:
Over-expression of certain miRNAs individually and in combination resulted in 13-27% increases in titer and 14-24% increases in qp in stably transfected pools. No significant alterations in proliferation rates were observed. 20 single cell clones were randomly selected from each of the 5 transfected pools for characterization. Statistical analyses showed significant differences in titer/qp between the high- and low-miRNA expressing single cell clones. The highest producing single cell clones exhibited ~100% increases in titer and qp compared to non-transfected cells. To our knowledge, this is the first report of enhancement of recombinant protein productivity by stable miRNA over-expression. The genes and cellular pathways targeted by these miRNAs specific to enhancing protein productivity are under investigation and will be reported.

CELL LINE DEVELOPMENT: IMPROVEMENTS THROUGH MODIFICATIONS AT AN EARLY STAGE

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KEY WORDS:
CHO / CHROMTAIN ELEMENTS / KNOCKOUT

BACKGROUND AND NOVELTY:
The generation of manufacturing cell lines is laborious and can require 100s-1000s of clones to be screened before identifying the final candidate. High through-put technologies have enabled more efficient screening but modifications in the early stages of cell line generation also have the potential to allow this.

EXPERIMENTAL APPROACH:
A number of approaches including the use of engineered cell lines, chromatin modifying elements, and the overexpression of “helper” proteins have been examined.

RESULTS AND DISCUSSION:
In the majority of cases these modifications can enhance colony number resulting in an improved distribution of the ”better” expressing clones, in some cases, the expression level of the clones also improves. Several of these approaches have been assessed at UCB and data will be presented on how the use of these has helped understand our cell line development process.

REF-B045

A ROBUST RMCE SYSTEM BASED ON A CHO-DG44 PLATFORM ENABLES EFFICIENT EVALUATION OF COMPLEX BIOLOGICAL DRUG CANDIDATES

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KEY WORDS:
CHO PRODUCER CELL LINES / GENE TARGETING / CELL ENGINEERING / RECOMBINANT GLYCOPROTEIN EXPRESSION / BIOLOGICAL DRUG EVALUATION

BACKGROUND AND NOVELTY:
Complex biologicals are typically produced in mammalian cell lines where CHO cells represent the working horse of the biopharmaceutical industry. In earlier biologics development stages there is often more than one potential candidate molecule against a specific target. A careful evaluation of those candidates is essential for choosing the optimal variant for further development leading to clinics and market. For studying potency, stability and manufacturability it is crucial to produce material for each individual candidate under comparable conditions. Hence, producer cells or process medium and conditions are known to influence important molecule features such as glycosylation patterns and activity. Using our CHO-DG44 platform candidate material can be produced in the same host cell and process background as used for pharmaceutical producer cell lines. Therefore, the molecular features of this material are expected to match with material that will be derived from a future producer cell line.

EXPERIMENTAL APPROACH:
For streamlining the generation of stable producer cell lines we have established an Flp-based RMCE system in our CHO-DG44 platform. Supported by a robust protocol gene replacement is fast and highly reproducible allowing a multiwell approach. Within only 4 weeks 1L fed-batch production is feasible that can be easily expanded to any desired scale. This typically yields in several 100 mg up to several g for monoclonal antibodies or other glycoproteins.

RESULTS AND DISCUSSION:
At significantly reduced effort the combination of our RMCE system and CHO-DG44 platform provides for multi-parallel cell line generation and production of high quality material that is ideally suited for candidate evaluation studies. In contrast to transient systems RMCE application allows for fast and repeated production in the process background of the future producer cell line at any time and scale.

REF-B046

RECOMBINASE MEDIATED CASSETTE EXCHANGE (RMCE) FOR ASSESSMENT OF GENE EFFECTS IN CHO CELLS WITH MINIMAL CLONAL VARIATION

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KEY WORDS:
CHO CELLS / RECOMBINASE MEDIATED CASSETTE EXCHANGE (RMCE) / FACS

BACKGROUND AND NOVELTY:
Metabolic engineering is mostly achieved by random integration of the gene of interest (GOI) into the genome. The resulting variation in clonal properties, caused by the chromosomal rearrangements that ensue, make it difficult to assess the influence of genes to be tested on physiology, as observed variations in clone behaviour may be caused by overexpression of the GOI, but just as well by random genetic rearrangements caused by transfection. We therefore developed protocols to transfer GOIs into pre-selected genomic locations, thus minimising clonal variation between the recombinant and the parental clone.

EXPERIMENTAL APPROACH:
A sortable reporter gene (CD4), flanked by Flippase Recognition Targets (FRTs) and having a leaky start codon is transfected into CHO cells. The leaky start codon reduces protein translation, and allows sorting for the highest productivities, while the FRT sites enable subsequent gene exchange. After repeated rounds of RMCE (to select for sites that allow reliable cassette exchange) and sorting for top producers by FACS, the stability of transgene expression is tested by removal of selection pressure, re-sorting and final sub-cloning. The established clones can be physiologically and metabolically characterised and serve as reference cell lines into which the GOI can be inserted without major genetic alterations. By using three start codons (ATG, CTG and TTG) the translation of the GOI can be controlled at 100%, 10% or 1% of the maximum expression level, to adjust for the desired concentration in cells.

RESULTS AND DISCUSSION:
Two rounds of cassette exchange (typically with an efficiency of 0.8%) using different reporter genes and FACS sorting for top producers were followed by long-term stability testing, where CD4 expression was shown to be stable. Exchange of the CD4 gene with the initial Neomycin against the GOI in combination with Blasticidin and sorting for the absence of CD4 enabled efficient establishment of recombinant cell lines for multiple genes within one month.

UTILIZATION OF RECOMBINASE-MEDIATED CASSETTE EXCHANGE (RMCE) TO DEVELOP STABLE CHO CELL LINES WITH DEFINED TRANSGENE EXPRESSION PROPERTIES

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KEY WORDS:
RECOMBINASE-MEDIATED CASSETTE EXCHANGE / RMCE / TARGETED GENE INTEGRATION / FLP / FRT

BACKGROUND AND NOVELTY:
The circumvention of the unpredictable effects of random transgene integration into the genome of host cells is of major interest to both industry and basic research. Several studies have shown that the respective integration site, in combination with the vector construct and promoter, has a significant impact on the transcription efficiency of the GOI (Gene Of Interest). The establishment of site-specific recombinases as straightforward tools to genetically engineer cells opened up the possibility to specifically target genomic sites with defined properties.

EXPERIMENTAL APPROACH:
The utilization of Recombinase-Mediated Cassette Exchange (RMCE) offers the possibility of exchanging the reporter gene for any other GOI and thereby integrating it at a pre-defined genomic locus with characterized expression properties. To achieve this, plasmids were designed which carry the reporter gene CD4, GFP or an IgG-antibody gene, flanked by two heterospecific FRT (Flippase Recognition Target) sites and alternative selection markers. Various stable CHO subclones were sorted with defined CD4 expression levels. Gene expression stability was examined over a period of two months and suitable clones were chosen for subsequent RMCE experiments using the site-specific recombinase Flpo.

RESULTS AND DISCUSSION:
In the course of this project cell banks of various stable CHO clones, ranging from low to high CD4 expression were established. An electroporation based protocol for successful gene exchange was generated, as well as a strategy for the efficient isolation of recombinant cell populations using a combination of chemical selection and flow cytometric cell sorting for absence of CD4 expression as the criterion for successful gene exchange. RMCE has proven to be a powerful tool, allowing generation of positive recombinant pools with defined expression levels within a four week period.

COMPARISON OF A CHIMERIC AND THE ENDOGENOUS MIR-221~222 - CLUSTER IN CHO CELLS

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KEY WORDS:
MICRORNA / CHO

BACKGROUND AND NOVELTY:
Today Chinese Hamster Ovary cells are the most commonly used mammalian cell factory for the production of recombinant therapeutic proteins. Their advantages compared to microbial cells are correct protein folding, a high secretion rate and human-like post-translational modifications. The main drawback is the time-space yield, which is at least tenfold lower than in microbial host systems. Many cell line engineering strategies were developed to overcome the major disadvantages of CHO cells. One approach is microRNA (miRNA) engineering which has the advantage over conventional cell line engineering approaches that miRNAs do not require the translational machinery. We investigated the impact of different miRNA-constructs on CHO cells.

EXPERIMENTAL APPROACH:
A chimeric miRNA cluster consisting of hairpin and flanking sequences of mmu-miR-155 (used as a standard plasmid for transfection of shRNAs) and the mature miR-221~222 sequences of CHO, and the endogenous stem-loops of the CHO-miR-221~222 cluster, which were amplified from genomic DNA, were cloned into the pcDNA6.2-GW/EmGFP-miR vector. CHO DUKX-B11 EpoFc 14F2 cells were transfected with the plasmid containing GFP and the respective miRNA construct, and the amounts of active GFP, GFP-mRNA and of the mature miR221 and miR222 were determined.

RESULTS AND DISCUSSION:
Though the same amount of plasmid was used for all transfections, and the GFP-mRNA were expressed at equal levels, the mature miR221-3p and miR222-3p of the endogenous cluster were two- to eightfold higher expressed relative to the negative control whilst the expression levels of the mature microRNAs of the chimeric construct did not show an increase. It seems that the characteristics of the hairpin structure are critically important for proper processing of microRNAs, which demonstrates the importance of using species specific sequences for microRNA engineering.

REF-B049

ENGINEERING THE ENERGY METABOLISM AND LACTATE PRODUCTION IN MAMMALIAN CELLS: DOWN-REGULATION OF THE WARBURG EFFECT

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KEY WORDS:
AA-CRYSTALLIN / ER STRESS / ER STRESS INDUCED APOPTOSIS

BACKGROUND AND NOVELTY:
Most common strategies to enhance the productivity of the therapeutic proteins in CHO cells include gene amplification methods and/or use of strong promoter. Whichever may be the method of choice, ultimately the cell's intrinsic protein synthesis and protein folding capacity becomes limited due to the perturbations in the endoplasmic reticulum (ER) function. Further accumulation of misfolded protein in the ER causes ER stress. However when the ER stress is unresolved and chronic, it leads to apoptosis. ER stress induced apoptosis is one of the setback in the CHO cells, limiting their productivity in culture.

EXPERIMENTAL APPROACH:
Extension of the cell viability in culture by delaying the process of ER stress induced apoptosis can efficiently improve the recombinant protein yield in CHO cells. a- crystallins, belonging to small heat shock protein family, functions as a regulators of ER stress induced apoptosis and provides protection against wide range of cellular stresses. The anti-apoptotic effects are due to the ability to bind the pro apoptotic molecules p53, Bax and Bcl-X(S) and thereby prevent their translocation to mitochondria during apoptosis.

RESULTS AND DISCUSSION:
In the present study CHO-Antibody producing (CHO-Ab) cells, were engineered to over express the aA-crystallin. The engineered clones exhibited a gradual viability loss and extension of the culture time of 24-36 hours in batch culture. The extension in the culture time translated to 53-62% improvement in specific productivity of CHO-Ab cells. These results suggest that over expression of aA- crystallin could be an efficient engineering strategy to enhance the culture performance and productivity.

REF-B050

INCREASING THE YIELD OF BIOTHERAPEUTIC RECOMBINANT ANTIBODIES FROM MAMMALIAN CELLS BY MANIPULATION OF THE SIGNAL SEQUENCE PEPTIDE

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KEY WORDS:
CHO CELLS / RECOMBINASE MEDIATED CASSETTE EXCHANGE (RMCE) / FACS

BACKGROUND AND NOVELTY:
The maximum yield of recombinant antibody achieved from industrially engineered mammalian CHO cells has increased greatly over the last 20 years, but in order to keep pace with increased pressure for production of these biotherapeutics, more specific engineering of cellular pathways needs to be addressed. This research seeks to gain a greater understanding of the secretory pathway within CHO cells, particularly with reference to how the N-terminal signal sequence can have an effect on the total antibody secreted. Although the signal sequence plays a vital role in the successful secretion of a recombinant antibody, it is not actually incorporated into the final product, making it an ideal candidate for engineering. Four endogenous CHO signal sequences, plus a non-endogenous N-terminal sequence, were compared to an antibody signal sequence in a transient CHO expression system. Growth curves, productivity measurements, mRNA analysis and the effectiveness of codon optimisation were all investigated in order to calculate correlations with product secretion. It is hoped that this work, plus future studies using identical signal sequences on other recombinant proteins, will allow us to begin to build a model for predicting the optimal N-terminal sequence to use with current and novel recombinant proteins in an industrial cell line.

CHARACTERIZATION OF INCREASED PYRUVATE DEHYDROGENASE ACTIVITY INDUCED BY DICHLOROACETATE IN HEK293 CELLS AT A CELLULAR SYSTEM LEVEL

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BACKGROUND AND NOVELTY:

Aerobic glycolysis, also known as the Warburg effect, is a metabolic phenotype characterized by cells preferring glycolysis and conversion of pyruvate to lactate over oxidative phosphorylation despite the presence of oxygen. The pyruvate dehydrogenase complex (PDH) plays a crucial role in this phenomenon as it controls how much pyruvate is converted to acetyl-CoA and hence unavailable for lactate formation. Aerobic glycolysis and decreased PDH activity is not only linked to various diseases such as cancer, glucose intolerance and neurodegeneration but is also observed in non-cancerous continuously proliferating cells such as cell lines used as production hosts in industrial applications. In this study we increased PDH activity and characterized the effects at a cellular system level using multiple omics technologies to further understand the importance of the pyruvate node and PDH activity for the cell.

EXPERIMENTAL APPROACH:

We increased PDH activity in Human embryonic kidney 293 (HEK293) cells using dichloroacetate (DCA) which indirectly activates PDH by inhibiting PDK, a PDH inhibitor. HEK293 cells were cultivated with two concentrations of DCA (5mM and 10mM) and a multi-omics approach was used to identify changes in the transcriptome, proteome, metabolome and fluxome induced by increased PDH activity.

RESULTS AND DISCUSSION:

DCA caused a reduction in PDH phosphorylation in HEK293 cells hence increasing its activity. We observed a concentration dependent decrease in glucose consumption and lactate production indicating a reduction in aerobic glycolysis with increasing DCA concentration. In addition, some amino acid production and consumption rates as well as intracellular metabolite concentrations were altered. For all used DCA concentrations changes in metabolism and in metabolic fluxes of central metabolic pathways were observed. The lower DCA concentration had no effect on cell growth and mRNA expression, while at high concentration cell growth decreased and changes in proteome and transcriptome were observed including the PDH inhibitor PDK, few enzymes of glycolysis and several other cellular pathways. These data give new insights on the effects of increased PDH activity on the cell as a whole.

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REF-C001

EFFECT OF AERATION STRATEGY AND BIOREACTOR ENVIRONMENT UPON CHO CELL PERFORMANCE

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KEY WORDS:
CFD / CHO / AERATION / APOPTOSIS / F-ACTIN

BACKGROUND AND NOVELTY:
The production of therapeutic proteins with complex post-translational modifications is typically performed with suspension adapted mammalian cells, in stirred tank bioreactors (STRs). In the STR agitation and aeration generate an environment in which cells are under continuous stress [1]. We use a validated computational fluid dynamics (CFD) model and a series of experimental approaches to characterise these stresses and the impact this has on the cells and product.

EXPERIMENTAL APPROACH:
Chinese hamster ovary cells were grown in batch and fed-batch mode in 5L STRs, for which the hydrodynamic environment was characterised by CFD. Cells were grown in chemical defined medium with and without shear protectant agent, using two aeration systems; direct gas sparging (0.03, 0.14 vvm) and a silicone membrane aeration system (SMAS) (0.03 vvm). Apoptosis analysis and cytoskeleton staining were performed throughout the culture and glycosylation profile determined by N-glycan analysis.

RESULTS AND DISCUSSION:
A bubble free environment was achieved via a SMAS and proved to be an efficient aeration system with comparable cell culture results to those obtained by direct gas sparging. Both aeration systems showed transitions from viable to late apoptotic populations and had reductions in F-actin intensity as the culture progressed. These effects were accentuated with direct gas sparging at a higher gas flow rate. Fed-batch mode delayed the onset of lethal and sub-lethal effects, extending culture duration and increasing product titre 2-fold. The glycosylation profile was not significantly affected by aeration conditions, with harvest time shown to have a greater impact [2]. CFD indicated that the hydrodynamic forces vary within the STR. The energy dissipation rates obtained from CFD simulations were higher than those previously reported [3]. Despite this the cells were capable of growing and producing mAb with good product quality but presented lethal and sub-lethal effects towards the end of culture.

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Acknowledgments:
Lonza Biologics are acknowledged for the provision of the cell line utilised in this work and the Mexican Council on Science and Technology (CONACyT) for funding.

REF-C003

RAPID ANALYTICAL TECHNIQUE TO ASSESS THE QUALITY OF CELL CULTURE MEDIUM

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KEY WORDS:
CELL CULTURE MEDIUM / MULTIVARIATE ANALYSIS / RAW MATERIALS

BACKGROUND AND NOVELTY:
SAFC strives to be the premier supplier of cell culture medium and has instituted multiple initiatives to better understand the raw materials that are utilized in cell culture medium. One initiative is the raw material characterization (RMC) program at SAFC that was initiated in 2009. The primary focus of this program is the individual screening of raw materials both analytically and biologically. One of the key learns from this program is that as a supplier of cell culture medium, the need to have a rapid method to assess the product quality is critical.

EXPERIMENTAL APPROACH:
A medium is consisted of numerous components that differ chemically, hence, multiple assays need to be run including amino acids, vitamins and trace elements. Spectroscopic techniques like NIR and Raman coupled with multivariate analysis (MVA) are being evaluated as a qualitative tool to evaluate culture medium as well as undefined raw materials like plant hydrolysates for differences in product quality and/or composition. These methods are rapid but non-specific and do not have the resolution to pick up trace components. Therefore there is a need to have a rapid and quantitative assay as a quality tool that can measure as many components as possible to verify media quality. A rapid ultra-high performance liquid chromatography-mass spectrometry (U-HPLC-MS) based method is assessed as a quantitative method for this purpose. Such a method in place coupled with MVA can help provide product of the highest quality and consistency.

RESULTS AND DISCUSSION:
Multiple cell culture media are evaluated using U-HPLC-MS. Some of the samples tested were deliberately misformulated to test the validity of the method in picking up missing components. This approach can provide a very reliable and quantitative tool in assessing medium quality. This presentation will highlight the multiple approaches SAFC is taking to ensure the best quality product is being delivered to the end user.

GLYCOPATH – A SOFTWARE TOOLBOX FOR THE IDENTIFICATION AND VISUAL ANALYSIS OF GLYCOSYLATION IN MAMMALIAN CELL CULTURES

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KEY WORDS:
GLYCOMICS / GLYCOSYLATION / CHO / BIOINFORMATICS

BACKGROUND AND NOVELTY:
Glycans are complex carbohydrate chains that play a key role in several structural and modulatory functions in cells. The glycosylation profiles of therapeutic recombinant proteins produced in mammalian cell lines are also known to directly affect their efficacy and viability. Despite the therapeutic importance of glycans, the identification and subsequent comparative analysis of glycan structures remains a bottleneck. This bottleneck is due to the lack of adequate software available for such analysis, resulting in a time spent performing manual analysis of glycosylation data. To help alleviate this shortcoming we have created a software pipeline to identify glycan structures from mass spectrum data and visually explore the resulting glycan distribution.

EXPERIMENTAL APPROACH:
We have used the MATLAB software package to carry out the data analysis and to create a user-friendly graphic user interface (GUI) for the convenience of users not familiar with MATLAB. The user can begin the data analysis starting from the raw mass spectrum file and optional experimental parameters used during the mass spectrometry experiment as input. After the glycans have been identified from the spectra they can be plotted onto an N-glycosylation biosynthesis network to display the distribution of glycan structures and usage of glycan biosynthesis enzymes. The software can also perform comparative analysis of enzyme usage between different production batches.

RESULTS AND DISCUSSION:
The software was used to analyze two samples of Chinese Hamster Ovary (CHO) cell lines – a wild type strain and a CHO Lec1 mutant strain. The automatic analysis was able to correctly identify all glycan structures that had previously been found through manual analysis, while also identifying key differences in enzyme usage between the two samples. Our software provides a complete pipeline from raw spectrum data to differential analysis of glycan samples, significantly reducing manual effort that has been needed.

HYDRODYNAMIC CHARACTERISTICS OF THE INDUSTRIAL STIRRED TANK BIOREACTOR

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KEY WORDS:
STIRRED TANK / CFD / GAS-LIQUID MASS TRANSFER / FLOODING OF THE IMP

BACKGROUND AND NOVELTY:
The good performances of stirred tank bioreactors are influenced, among other factors, by the agitation and aeration conditions set and by their geometric characteristics. These factors determine the flow patterns on the equipments. To meet the needs of knowledge on flow patterns and other variables related to them in the industrial bioreactor for animal cell culture, this paper proposes the use of Computational Fluid Dynamics (CFD) and experimentation and to obtain novel information on the hydrodynamics in the operating conditions.

EXPERIMENTAL APPROACH:
The bioreactor studied, whose workload is 1100 L (Bioengineering AG, Switzerland) has two impellers coupled to the same shaft. The bottom impeller is a Rushton turbine blades 6 to drive the fluid radially. While the upper impeller has a marine propeller with 3 blades oriented to drive the fluid axially downwardly.

RESULTS AND DISCUSSION:
We propose a mathematical model using the CFD tool, with which velocity fields comparable to those reported in the literature for a bioreactor with the same geometry as the one studied were obtained. By experimentation the time of mixing and the residence time distribution (RTD) were determined in the operating condition of the bioreactor (without aeration), and a flow pattern of perfect mix for the liquid phase was obtained. Also the mixing time was determined by CFD reaching a good approximation to that obtained experimentally. The operation of the bioreactor with air was characterized using a factorial experimental design 3^2 and the results obtained showed that the coefficient of volumetric oxygen transfer (kLa) depends on the variables stirring speed (N) and air flow rate (Qa).Using published correlations in the literature it was concluded that the phenomenon of flooding of the impeller occurs in the operating condition. This phenomenon was also observed by means of a mathematical model. The relative errors among the values of kLa obtained experimentally and by CFD are in the order of 20% which is acceptable.

REF-C006

GENERATION OF PATHWAYS IN METABOLIC NETWORKS: A DYNAMIC APPROACH USING COLUMN GENERATION AND EXTRACELLULAR MEASUREMENTS

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KEY WORDS:
METABOLIC PATHWAYS / ELEMENTARY FLUX MODES / EXTREME PATHWAYS / OPTIMIZATION / METABOLIC FLUX ANALYSIS

BACKGROUND AND NOVELTY:

In recent years more detailed metabolic networks of cells are being defined. These larger networks need robust methods to find feasible pathways [1]. Elementary modes (EMs) and extreme pathways (EPs) are two concepts used for this purpose. The EMs of a network can be enumerated using the program Metatool [2]. However, the number of EMs increases with increasing network dimension, making the enumeration of all feasible EMs prohibitive [3]. Identifying only relevant EMs with respect to experimental data could facilitate interpretation and modeling of metabolic states. This has been investigated in the framework of EP analysis, by applying singular value decomposition to determine the most important EPs to a cell from the whole set of EPs [4]. In the present work, the aim was to develop a method that finds only the most relevant pathways with respect to data, enabling the use of larger metabolic networks. We present a method based on a column generation technique that finds pathways in a dynamic fashion, while taking extracellular metabolite measurements obtained in cell culture experiments into consideration.

EXPERIMENTAL APPROACH:

We minimize the norm of the difference between extracellular rate measurements and calculated flux through macroscopic pathway reactions in a metabolic network. Starting with only the network, pathways that improve the norm are generated iteratively until no new pathway can further minimize the norm. Hence only the most relevant pathways are found.

RESULTS AND DISCUSSION:

The main result of our work is a method that finds pathways in a network guided by experimental data. The usefulness of the approach is demonstrated using data representing metabolic states obtained in cell culture with different media. Networks of varying sizes are used and the results compared to those obtained with Metatool. Our approach has the potential to find relevant EPs or EMs of larger networks, opening up the possibility for pathway analysis and modeling with larger networks than what has been possible before.

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OTHER INFORMATION

This project was performed as a collaboration between the School of Biotechnology and the Department of Mathematics at the Royal Institute of Technology (KTH).

REF-C007

QUANTITATIVE ANALYSIS OF GLYCOLYSIS IN MDCK CELLS DURING CELL GROWTH, MEDIUM REMOVAL AND MEDIUM ADDITION

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KEY WORDS:
MDCK CELL / GLYCOLYSIS / MATHEMATICAL MODEL / INTRACELLULAR METABOLITES

BACKGROUND AND NOVELTY:

Like many other continuous cell lines, adherently growing Madin Darby canine kidney (MDCK) cells gain most of their cellular energy from glycolysis. The cells typically grow exponentially until either the available surface or the concentration of extracellular metabolites limit proliferation. Although much is known about the relation between growth and metabolite use, the dynamics in intracellular enzyme reaction rates and metabolite pools of the respective metabolic pathways are still poorly understood.

EXPERIMENTAL APPROACH:

We developed a kinetic model for glycolysis, which describes enzyme activities as a function of metabolite levels. Therefore, MDCK cells grown in 6 well plates were first deprived of glucose by rapid withdrawal of the medium and addition of buffer. After 2 h, the buffer was replaced with fresh medium. Changes in intracellular metabolite concentrations were quantified by anion-exchange chromatography and mass-spectrometry. Additionally, maximum in vitro enzyme activities were monitored. The established model was then combined with a cell growth model considering metabolite use as well as changes in cell diameter.

RESULTS AND DISCUSSION:

The combined model fits the dynamics of the five measured intracellular metabolite concentrations during medium removal as well as during medium addition. For these extreme cases it indicates a strong interconnection of glycolysis with the pentose phosphate pathway. Simultaneously, the combined model describes the dynamics of glycolysis during cell growth and suggests that glucose carriers exert a high influence on intracellular metabolite pools and flux rates under typical cultivation conditions. Overall, the approach enables the characterization of the in vivo kinetics of enzyme-catalysed reactions and allows elucidating intracellular metabolite turnover, cellular energy generation and precursor supply of adherently growing mammalian cells.

GENEDATA BIOLOGICS – AN INTEGRATED DATA MANAGEMENT PLATFORM FOR BIOPHARMACEUTICAL R&D

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BACKGROUND AND NOVELTY:

The discovery and development of biopharmaceuticals is a complex and time-consuming endeavor. The management and interpretation of data generated in the biologics R&D process are key bottlenecks in making biopharmaceutical R&D more efficient.

EXPERIMENTAL APPROACH:

Genedata Biologics™ is an enterprise software platform that supports the entire biologics R&D process including antibody screening, protein engineering, as well as biologics expression, purification, and analytics. The focus of the Genedata Biologics platform is to support scientists in their daily R&D operations. The system helps to simplify and streamline laborious, manual processes such as cloning or instrument operation, resulting in increased efficiency and throughput, as well as in improved quality of results through reduction of ad-hoc and error-prone sample and data handling.

RESULTS AND DISCUSSION:

Genedata Biologics is the result of a concerted five-year development program in close collaboration with leading international players in biologics R&D. Here, we present concrete use cases including antibody phage-display, screening automation, affinity maturation, design and evaluation of engineered molecules, as well as high-throughput expression, purification, and analytics of novel biologics candidates.

QUANTITATIVE ANALYSIS OF CLONE PERFORMANCE THROUGH AT LINE ANALYSIS OF CELLULAR METABOLISM

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KEY WORDS:

BIOPROCESS ANALYSIS / CLONE PERFORMANCE / METABOLIC ANALYSIS / COMPUTATIONAL BIOTECHNOLOGY

BACKGROUND AND NOVELTY:

A fast, quantitative and comparative clone performance evaluation represents an essential tool for efficient and objective selection of the best production clones. In this contribution we introduce a newly developed tool that automatically processes fermentation data by a direct connection to process data warehouses and provides comparative evaluations of the cellular performance with site-independent access.

EXPERIMENTAL APPROACH:

On the basis of extracellular measurements of carbon sources, amino acids, cell number and product, the platform automatically identifies physiologically distinct process phases. For each of these phases, intracellular flux distributions including statistics are calculated. This procedure can be repeated for large numbers of fermentation runs (1,000+) in an automated fashion. Processes were compared both at the level of individual process phases and regarding overall metabolic performance with each other. Here, we demonstrate the application of the computational tool to comparing the metabolic performance of Chinese hamster ovary (CHO) clones.

RESULTS AND DISCUSSION:

Fermentation data from fed-batch runs of Chinese hamster ovary (CHO) cells was processed to determine process phases and intracellular flux distributions. Carbon fluxes were visualized by the tool along with nitrogen fluxes and cellular energy usage in the form of adenosine triphosphate (ATP) at the pathway level. Animation by specific pathway maps provided an intuitive access to intracellular fluxes. This enabled us to quickly identify, e.g., variations in ATP requirements for maintenance between clones exhibiting different performance. Moreover, we could trace and quantify different intracellular sources of ammonium formation during the course of individual fermentations. In summary, we illustrate how the comparative quantification of cellular metabolism contributes to (i) accelerated selection of the best production clones and (ii) to a significantly improved physiological expertise.

REF-CO10

THE CHANGING DIELECTRIC PROPERTIES OF CHO CELLS CAN BE USED TO DETERMINE EARLY APOPTOTIC EVENTS IN A BIOPROCESS

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KEY WORDS:
DIELECTROPHORESIS / CELL CULTURE / APOPTOSIS / CAPACITANCE / FLOW CYTOMETER

BACKGROUND AND NOVELTY:
A prototype dielectrophoretic (DEP) cytometer has been developed at the University of Manitoba to analyze individual CHO cells subjected to a radiofrequency actuator and detector in a narrow bore capillary. Cell samples during the course of a bioreactor run show distinct shifts in the dielectric properties corresponding to loss of cell viability. Discrete cell sub-populations can be identified during apoptosis and can be correlated with alternative measurements by fluorescent markers and a cell population-based capacitance probe.

EXPERIMENTAL APPROACH:
In our study five different and independent methods were compared for the determination of cell density and/or cell viability. These included the particle counter, image analyzer with trypan blue exclusion, an on-line capacitance probe, off-line flow cytometer apoptosis kits and a prototype DEP cytometer. The multiple methods of cell monitoring were applied to the culture of a CHO cell line grown in a 3L bioreactor and compared over the course of a bioprocess.

RESULTS AND DISCUSSION:
Our results show that the various on- and off-line techniques gave similar values during exponential phase and measurements diverged only at the point of highest cell density. Fluorescent flow cytometry was used to investigate this divergence: the intermediate stage apoptosis assay agreed with those obtained by the bulk capacitance probe and the early stage apoptosis assay viability measurements correlated well with the DEP cytometer while image analysis with trypan blue provided significantly higher estimates of cell viability. With this novel DEP technique we have established that the trajectory of viable CHO cells in a narrow bore capillary, subjected to a radiofrequency actuator, can be used to distinguish viable from non-viable cells. Also, sub-populations of cells can be identified that are associated with a progression through the stages of apoptosis. This opens up the possibility of monitoring the incremental changes that occur during cell growth and death in bioreactors.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-CO11

INTACT CELL MALDI MASS SPECTROMETRY BIOTYPING FOR "AT-LINE" MONITORING OF APOPTOSIS PROGRESSION IN CHO CELL CULTURES

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KEY WORDS:
CHO / APOPTOSIS / ICM-MS / AT-LINE MONITORING

BACKGROUND AND NOVELTY:
Mammalian cell cultures, especially Chinese Hamster Ovary (CHO), are the predominant host for the production of biologics. Despite considerable progress in industry and academia alike, particularly in the field of process monitoring there is still a need for integrated methods analysing e.g. the physiological state of cells. Intact cell MALDI mass spectrometry (ICM-MS) biotyping, a method used successfully in the field of clinical and environmental microbiology, is getting more attention in the context of mammalian cell cultivation. The identification of specific mass spectrometric signatures for cell physiological states using ICM-MS biotyping as reported here could be a promising tool for CHO culture monitoring and has to our knowledge not been published elsewhere.

EXPERIMENTAL APPROACH:
We use a Bruker Daltronik AutoFlex to investigate suspension cell CHO cultures. Samples for at-line analysis were prepared from as little as 2500 cells (Munteanu et al. 2012) [1]. Batch cultures were monitored via MS and reference analytics in order to investigate progression of apoptosis during cultures. Experimental aim was to establish characteristic MS patterns indicative for different apoptotic states with emphasis on detection of early apoptosis.

RESULTS AND DISCUSSION:
It was possible to identify specific apoptosis related MS patterns - including 20-30 m/z values - for the CHO cell lines analysed. Furthermore, MS analysis of bioreactor-like batch cultivations showed for many of these m/z values identified as "apoptosis-specific" already at early cultivation time-points slight but reproducible changes in signal intensity. Using the software ClinProTools (Bruker Daltonik) it was possible to develop a classification model, which allows the reliable discrimination of unknown samples regarding their cell physiological state (vital, apoptotic, necrotic). This procedure, based on simple and robust sample preparation and subsequent MS analytics, might be a first step towards application in "at-line" process monitoring.

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REF-C012

STANDARDIZED ONLINE BIOMASS MEASUREMENT IN SINGLE USE FERMENTATION

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KEY WORDS:
SU BIOMASS SENSOR / RADIO FREQUENCY (RF) IMPEDANCE METHOD / IN-SITU CELL DENSITY DETECTION / PROCESS ANALYTICAL TECHNOLOGY (PAT) TOOL / PROCESS MONITORING AND CONTROL

BACKGROUND AND NOVELTY:
According to various studies in 2012, the detection of biomass is one of the most requested parameters in industrial cell cultivation. The knowledge of the biomass progress during a fermentation process gives deeper process knowledge and control and helps to define harvest or infection points. Offline methods like visual cell counting or semi-automated systems still dominate the biomass detection in industrial cell cultivation. But these offline methods based on taking a representative sample cannot monitor the process continuously. The radio frequency (RF) impedance method for online in-situ detection of viable biomass has already become well established in biopharmaceutical applications using traditional reusable fermenter equipment. On the other hand, industrial cell cultivation tends more and more to single use (SU) fermentation equipment. This presentation shows test results of the first standardized online biomass measurement solution for SU fermenter systems which is fully integrated into the standard fermenter control system and tailored to the SU fermentation bags.

EXPERIMENTAL APPROACH:
Sartorius BioPAT®ViaMass biomass sensors integrated in different CultibagRM were used for the experiments with rocking motion fermentation. These systems use the RF impedance method to determine the biomass. The influence of the rocking motion and rocking parameters on the detection signals were investigated and optimization strategies applied. Accordingly, the cultivation of different cell lines were continuously monitored with the online system.

RESULTS AND DISCUSSION:
The rocking motion of the fermentation system causes signal fluctuations due to the variation of liquid level over the sensor. Appropriate optimization strategies could be found for the different rocking motion parameters. Using this, the biomass evolution could successfully be monitored continuously in the cell cultivation experiments. The performance of the standardized online biomass detection system for SU fermentation will be presented and discussed.

REF-C013

ONLINE GLUCOSE-LACTATE MONITORING AND CONTROL IN CELL CULTURE BIOPROCESSES

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KEY WORDS:
ONLINE GLUCOSE MONITORING / CONTROL

BACKGROUND AND NOVELTY:
Conventional biopharmaceutical manufacturing is characterized by validated process steps and extensive lab testing procedures. The FDA PAT-Guidance recommends the use of potential for improving development, manufacturing, and quality assurance through innovation in product and process development, process analysis and process control. Measurement of glucose, as a major nutrient during cell cultivation, has a key role for controlling the status of the cultivation process. Together with the amount of lactate and additional process parameters, like pH and DO, it gives the possibility to calculate specific consumption rates of nutrients. The user gets information about the status of the culture and of the cells.

EXPERIMENTAL APPROACH:
The on-line analyzing system BioPAT®Trace covers the different demands of long-term cell culture cultivations in different scales such as small volume cultivations and FDA-validated large scale productions. The sterile sampling systems based on filtration, dialysis or ContiTRACE disposable probes provide the perfect solution for reliable on-line sampling in bioreactors and biodisposables applied in industrial and laboratory facilities.

RESULTS AND DISCUSSION:
BioPAT®Trace is a dual-channel analyzer for the simultaneously measurement of glucose and lactate which is based on an enzymatic detection of the two analytes. Special attention has been paid to the ease of use and hygienic issues related to cGMP environments. The system follows the plug & plays principle, can be fully integrated into all facility environment scenarios and is compliant with all relevant regulatory guidelines. Integrated in an automation platform enabled with a 2 point glucose controller, e.g. as part of an S88 recipe module of the BioPAT®MFCS SCADA system, it is possible to realize a fully automated control loop for any kind of cultivation process. This will be shown with different cell lines and cultivation strategies.

REF-C014

A METHOD FOR ADHERENT CELL CULTURE MONITORING BASED ON PHASE CONTRAST MICROSCOPY IMAGE PROCESSING

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KEY WORDS:
MONITORING / CONFLUENCY / IMAGE PROCESSING / QUANTITATIVE / ADHERENT CELLS

BACKGROUND AND NOVELTY:
Unlike their suspension counterparts that benefited from rapid advances in instrumentation, adherent cell cultures remain difficult to characterize. Confluency, although widely used, is subjective and qualitative. Assays requiring cells to be detached prior to analysis potentially offer quantitative information but are limited to end-point analysis and lead to the loss of key culture characteristics such as spatial distribution and cell morphology. These limitations preclude the collection of time-course data and prevent the determination of valuable process kinetics. We propose the use of phase contrast microscopy (PCM) image processing to determine culture confluency in a quantitative and systematic manner. This approach is non-invasive and can be applied to adherent cell culture monitoring.

EXPERIMENTAL APPROACH:
All image processing algorithms were developed using MATLAB and C++. Experimental validation was carried out using mouse embryonic stem cells (mESC), Chinese Hamster Ovary cells (CHO) and human neuroblastoma cells (NB).

RESULTS AND DISCUSSION:
We present a method for automated confluency estimation (MACE) based on PCM image processing. A novel algorithm enabled the accurate and precise detection of cell contours by correcting for the so-called halo artifacts that are inherent to PCM. Confluency of a single image was computed in less than a second. When using 20 random images per culture (culture area of 9.6cm2), MACE was 3.6-fold more precise than experienced human researchers while maintaining comparable analysis times. We demonstrated non-invasive monitoring of a wide range of mESCs responses, including proliferation, growth arrest, cell death and transient morphological changes. MACE is quick, accurate, accessible (graphical user interface) and readily usable in any cell culture laboratory equipped with a phase contrast microscope. As such, it could lead to a standardized metric for adherent cell culture characterization and be used as a basis for the establishment of robust experimental protocols.

REF-C015

INSIGHTS INTO MONITORING CHANGES IN THE VIABLE CELL DENSITY AND CELL PHYSIOLOGY USING SCANNING, MULTI-FREQUENCY DIELECTRIC SPECTROSCOPY

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KEY WORDS:
DIELECTRIC SPECTROSCOPY / BIOREACTORS / APOTOSIS

BACKGROUND AND NOVELTY:
Real-time bioprocess monitoring is fundamental for maximizing yield, improving efficiency and process reproducibility, minimizing costs, optimizing product quality, and full understanding of how a system works. The FDA's Process Analytical Technology initiative (PAT) encourages bioprocess workflows to operate under systems that provide timely, in-process results. At the same time the demand for ever increasing supplies of biological pharmaceuticals, such as antibodies and recombinant proteins, has fueled interest in streamlined manufacturing solutions.

EXPERIMENTAL APPROACH:
Bioreactors that are monitored continuously and in real-time offer the advantage of meeting current and future supply demands with biological product of the utmost quality and safety, achieved at the lowest overall cost and with least risk. This paper will focus on how one research groups in has used scanning multi-frequency dielectric spectroscopy to comparatively profile multiple bioreactor runs and elucidate fine details concerning cell viability and mechanism of cell death.

RESULTS AND DISCUSSION:
The cellular information observed has not been available through other technologies. The presentation will also focus on how the technology can also be applied to Single use Bioreactors in a cGMP environment and on samples down to 1ml volume.

UNDERSTANDING CELL BEHAVIOR IN CULTIVATION PROCESSES - A METABOLOMIC APPROACH

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KEY WORDS:
METABOLOMICS / CHEMOSTAT / HUMAN CELL LINE / BIOCHEMICAL PATHWAYS / SYSTEMS BIOLOGY

BACKGROUND AND NOVELTY:
During a cultivation cells undergo a tremendous change in their metabolism when shifting from one state to another or when process parameters are changed. This leads to changes in growth behavior and productivity. The metabolism of animal cells is much more complex compared to microorganism such as E.coli. To understand the changes in intracellular metabolite concentrations and their impact on cell performance we used a systematic approach. By employing the chemostat mode at different steady state conditions we investigated the alterations of the concentrations of key metabolites during cultivations of a human production cell line.

EXPERIMENTAL APPROACH:
A recombinant human cell line (AGE1.hn AAT, Probiogen AG, Berlin) was cultivated in a 2 liter fully controlled bioreactor running continuously in chemostat mode at different set points. Samples were taken at least daily for monitoring of cell density, viability and substrate concentrations (extracellular). After reaching steady state conditions at each set point cell samples were taken und prepared for intracellular metabolite analysis. For metabolic quenching the established fast filtration procedure [1] was used. The metabolites were extracted from the cells and analyzed by LC-MS using HILIC chromatography and Electrospray-Triple Quad MS/MS detection.

RESULTS AND DISCUSSION:
The cells were cultivated in chemostat mode in two different chemically defined media at three dilution rates (and three pH values) for more than 70 days. Most of the metabolites of the glycolysis and TCA pathway could be quantified as well as the nucleotides. The intracellullar metabolite concentrations varied significant with changing dilution rates and pH values. The pyruvate concentration declined with increasing dilution rate and decreasing pH value. While the concentration of almost all nucleotides dropped with increasing dilution rates they were more or less stable at changing pH values. More data are given and possible consequences for process control will be discussed.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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NEW INSIGHTS IN REGULATIONS OF SODIUM BUTYRATE TREATED CHO CELLS BY LABEL-FREE MASS SPECTROMETRIC PROTEIN QUANTIFICATION

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KEY WORDS:
CHINESE HAMSTER OVARY / SODIUM BUTYRATE / PROTEOMICS / CELL REGULATION / HISTONES

BACKGROUND AND NOVELTY:
Many approaches have been applied to identify regulatory processes by which an impact on cellular events for the optimization of productivity of CHO (Chinese hamster ovary) cells is given. The addition of sodium butyrate (NaBu) increases productivity, but also reduces growth of CHO cells. Though several gel-based proteomics approaches were performed in this context, the results gave only limited insight into exact mechanisms of increased productivity. Hence, we have chosen a gel- and label-free proteomics experiment to further investigate effects of NaBu in CHO cells. The results give novel insights in the regulation background after butyrate treatment.

EXPERIMENTAL APPROACH:
Cultivation of CHO cells was carried out in shaking flasks in 12 replicates. 6 replicates were treated with 2 mM NaBu after 64 h, and samples were taken after further 24 h. For the label-free mass spectrometric (LF-MS) approach, identification and quantification of digested total protein extracts were carried out in separate nanoLC-MS runs - with a rapid LC system coupled to a high resolution MS. For differential analysis, quantification data were linked in ProteinScape-software to identified peptides by automatic retention time alignment, and a t-test was performed to determine significantly regulated proteins ($p < 0.05$).

RESULTS AND DISCUSSION:
CHO-cells exhibited an 1.7 fold increased average specific productivity after addition of NaBu, but in contrast reached only 63 % of viable cell density (VCD) compared to untreated cells. Quantitative evaluation of the LF-MS proteomics approach resulted in 121 significantly regulated (p 0.05) out of 601 identified proteins. Next to other, protein abundances out of the functional classes of energy metabolism, cell cycle & growth, cytoskeleton, protein transport and nucleotide metabolism were significantly decreased after NaBu treatment. Interestingly, 11 of 13 proteins involved in proteolysis were down regulated, and altered levels of the 4 core-histones indicate a remodeling of chromatin structure.

REF-C018

ADVANCED OFF-GAS MEASUREMENT USING PROTON TRANSFER REACTION MASS SPECTROMETRY TO PREDICT CELL CULTURE PARAMETERS

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KEY WORDS:
OFF-GAS / MASS SPECTROMETRY / VOLATILE ORGANIC COMPOUND

BACKGROUND AND NOVELTY:
Mass spectrometry is a well-known technology to detect O2 and CO2 in the off-gas of cell culture fermentations. In contrast to classical mass spectrometers, the proton transfer reaction mass spectrometer (PTR-MS) applies a very soft ionization strategy and therefore the spectra show less fragments and are easier to interpret. This gave us the possibility to identify other compounds in the bioreactor off-gas stream and beside O2 and CO2. In our study we applied the PTR-MS technology for the first time to monitor volatile organic compounds (VOC) and to predict cell culture parameters in an industrial mammalian cell culture process.

EXPERIMENTAL APPROACH:
CHO cell culture processes producing a recombinant protein were conducted in a modified 7L glass bioreactor. For the study a PTR-MS with a QMS422 quadropole for mass separation with a secondary electron multiplier detector was used and masses ranging from 18 to 200m/z were measured. To correlate the PTR-MS data with cell culture parameters partial least square (PLS) models were used.

RESULTS AND DISCUSSION:
We created partial least square (PLS) models to predict the important cell culture parameters viable cell density, titer and glutamine with the PTR-MS data as input parameters. The best prediction could be achieved for the titer with a correlation coefficient R2 of 94% using five latent variables. The prediction of glutamine was poor, especially the predictive power (Q2 of 62%), which was not surprising since glutamine itself is not a volatile compound. Hence we created a PLS model for the specific glutamine uptake and the prediction for this parameter was better (Q2 of 82.2%). This indicated that volatile compounds are produced when important substrates such as glucose / glutamine are consumed. We demonstrated that the PTR-MS technology can provide important additional information about cell culture processes.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-C019

TOWARDS AN ANALYTICAL PLATFORM FOR METABOLIC STUDIES IN ADHERENT AND SUSPENSION CELLS: INTRACELLULAR METABOLITE CONCENTRATIONS AND MAXIMUM ENZYME ACTIVITIES

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KEY WORDS:
INTRACELLULAR METABOLITES / ENZYME ACTIVITIES / SUSPENSION CELLS / ADHERENT CELLS

BACKGROUND AND NOVELTY:
Assays to measure intracellular metabolite concentrations (IMC) as well as enzyme activities (EAs) have been successfully established for adherent MDCK cells. Nevertheless, the most critical step remain the choice of suitable assay conditions, like quenching and extraction solutions as well as the adaptation of parameters for measurement of EAs which are cell line-dependent. Here, we describe results obtained for a screening of various extraction methods for quantitation of IMC in adherent cells, and the adaptation of the existing protocol for monitoring growth of suspension cells. In addition, a platform established for EA measurements was evaluated for monitoring the metabolic status of two different suspension cell lines (AGE1. HN.AAT, HEK.293) and primary human leukemia cells.

EXPERIMENTAL APPROACH:
Measuring IMC via liquid chromatography and mass spectrometry, several methods involving culture medium removal and sample purification, use of a sampling probe coupled with a heat exchanger device for direct sampling and quenching during bioreactor cultivation, and impact of quenching solution were tested. The existing EA assays were adapted for each enzyme and cell line by screening for optimal assay parameters such as pH, assay time, cell and substrate concentration.

RESULTS AND DISCUSSION:
Cold 0.9% NaCl (4°C) was selected as an optimal quenching solution as it prevented cell damage and effectively slowed down turnover of metabolites. Use of a heat exchanger during sampling enabled fast and reproducible quenching and guaranteed high cell viabilities. Based on optimized protocols, data sets containing more than 25 IMs and 27 EAs from glycolysis and citric acid cycle, including cellular nucleotides are now available for adherent and suspension cell lines. Overall, the expanded assay platform has improved our understanding of basic cell growth properties, offers new possibilities for data analysis and mathematical modeling and allows identification of options for improvement of biotechnical or medical approaches.

ASSESSMENT AND QUANTIFICATION OF GENOMIC REARRANGEMENTS IN CHO PRODUCER CELL LINES

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KEY WORDS:
CHINESE HAMSTER OVARY CELLS

BACKGROUND AND NOVELTY:
The adaptability of Chinese hamster ovary (CHO) cells, the ease of genomic manipulation and their capacity to produce high quality human-like glycosylated proteins has made them the most frequently used cell line for industrial production. However, chromosomal rearrangements in CHO cells occur at a significantly higher frequency than in comparable immortal cell lines. While this might be the source of easy adaptation and the occurrence of extraordinarily high producers, it also requires a large number of cells to be tested during screening to isolate cells with sufficiently stable properties. Despite the impact on cell line development, there are few methods established to quantify and assess genomic instability in CHO cells, except for laborious and low throughput methods such as karyotyping and Fluorescence in situ hybridisation.

EXPERIMENTAL APPROACH:
A method used in plant breeding and cancer research to determine genomic changes and variation was adapted for use in CHO cells: Amplified Fragment Length Polymorphism (AFLP). Genomic DNA is isolated and digested using two restriction enzymes that recognize relatively long sequences consisting of at least 8 bp. Fragments are ligated to predefined sequences that serve as primers for subsequent PCR. By using primers with one, two or three additional nucleotides, the number of bands obtained by electrophoresis can be controlled.

RESULTS AND DISCUSSION:
The method results in defined patterns of electrophoretic bands that are analysed on a bioanalyzer. The profiles of different cell lines can be easily compared to determine the degree of genomic differences between CHO cell strains or subclones. In addition, the method can be used to describe the rate of genomic rearrangements of a given cell line over time and detects genomic rearrangements faster than observation of cell behaviour which is currently used. The method can be performed with standard equipment and is medium throughput, so that it can be used on a routine basis for multiple strains and subclones.

SINGLE STEP CLONING-TITRATION METHOD: ACCELERATING THE DEVELOPMENT AND ENGINEERING OF HIGH-TITER VIRUS PRODUCING CELL LINES

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KEY WORDS:
VIRUS TITRATION / HIGH-THROUGHPUT / SPLIT-GFP

BACKGROUND AND NOVELTY:
Mammalian cell line development for the production of complex biopharmaceuticals is a laborious and time-consuming work due to the need of screen for high-titer clones. The challenge increases for virus producer stable cell lines as viral components are usually split in several expression cassettes for engineering and/or safety purposes. Herein, we describe a novel method for fast screening of high-titer virus producing clones by merging cloning and titration.

EXPERIMENTAL APPROACH:
The method makes use of split-GFP, a green fluorescent protein separated into 2 fragments – S10 and S11 – which fluoresce only upon transcomplementation. A cell population producing infectious virus with a S11 transgene is cloned and co-cultured with a target cell line harboring the S10 fragment. S11 viruses produced by the clone infect the target cells and reconstitute the GFP signal. Only the clones yielding high signal are isolated, avoiding cell expansion, cell banking and posterior growth/titration studies, wasteful for the majority of the (low titer) clones.

RESULTS AND DISCUSSION:
The method was validated by establishing a retrovirus producer from a nude cell line reducing a 1 year process of sequential transfection/screening of 3 viral expression cassettes to 3 months. It was additionally used in metabolic engineering to identify rare high-producing phenotypes, among more than 300 clones. This performance was achieved in 2 weeks, whereas by the traditional analysis over 1 year would be required. It was established using a 96-well fluorometer plate reader, giving a manual throughput of approximately 500 clones per analysis; if coupled to an automated device, this can be increased by several orders of magnitude. Moreover, it is directly applicable to stable cell lines producing other types of infectious virus and, with the proper modifications, extendable to transient systems. We expect this approach to contribute to the progress of vaccine and gene therapy vector fields by accelerating cell line development and engineering.

REF-C022

REAL-TIME MONITORING OF CELL GROWTH AND METABOLIC SHIFT IN MAMMALIAN CELL-CULTURES USING DIELECTRIC SPECTROSCOPY AND CHEMOMETRICS

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KEY WORDS:
DIELECTRIC SPECTROSCOPY / CHEMOMETRICS / MONITORING / CHO / CELL GROWTH

BACKGROUND AND NOVELTY:
In mammalian cell culture processes of producing therapeutic proteins, changes in viable cell density is one of the crucial parameters in monitoring and diagnosing metabolic status of cell growth and protein production. Among the various kinds of real-time process analytical techniques, dielectric spectroscopy is one of the reliable real-time monitoring tools for the VCD estimation due to its simplicity, robustness and accuracy. Dielectric spectra measures the permittivity of the culture broth at multiple frequencies in a real-time manner, and can be well correlated to the cell density. However, the drawback is that an estimation of VCD is often deteriorated after the growth phase due to the abrupt changes in the physiological state of cells as well as the alteration in the electrochemical properties of culture media.

EXPERIMENTAL APPROACH:
In this study, the dielectric spectroscopy was applied to the bioreactor operations with CHO cells, and the chemometrics was employed to improve the VCD estimation from the permittivity measurements. Principal components analysis was utilized to investigate the permittivity pattern. Partial least squares (PLS) regression technique was modified by adopting the locally-weighted regression schemes in order to accommodate the different correlation structure in estimating the VCD from the permittivity measurements.

RESULTS AND DISCUSSION:
The estimation performance of the PLS-based estimation models was compared to the conventional approach of utilizing theoretical equation of Cole-Cole distribution, and the results indicated that the modified PLS-based approaches outperformed the Cole-Cole equation-based one as well as the unmodified PLS, especially after the growth period. The proposed approach was implemented within the framework of multivariate statistical control (MSPC) for consistent operation of biopharmaceutical manufacturing processes which poses great potential in determining optimal harvesting time for consistent therapeutic protein production.

REF-C023

CELL SURFACE-FLUORESCENCE IMMUNOSORBENT ASSAY FOR REAL-TIME AND FEMTOGRAM-LEVEL DETECTION OF HYBRIDOMA WITH MOST EFFICIENT ANTIBODY SECRETION

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KEY WORDS:
SINGLE CELL-BASED BREEDING / HIGH-THROUGHPUT SCREENING / BIOMOLECULE-SECRETING CELLS / AUTOMATED SINGLE CELL ANALYSIS AND ISOLATION SYSTEM

BACKGROUND AND NOVELTY:
Cells secreting biomolecules, such as Chinese hamster ovary (CHO) cells and hybridomas, have been important resources for sustaining bio-industries. The screening and breeding of promising cells require several weeks to propagate a substantial number of cells to form colonies from single cells for evaluation by the conventional assays, but lack high-throughput performance in time and colony numbers. Therefore, it has been expected to develop novel methods for identifying single cells secreting higher amounts of biomolecules in real-time and in a nondestructive manner without colony formation.

EXPERIMENTAL APPROACH:
In this study, a nondestructive fluorescence-labeling system (a cell surface fluorescence immunosorbent assay (CS-FIA)) was established for the high throughput selection of cells secreting higher amount of biomolecules from cell library. The assay consists of four steps: 1) lipid-labeled antibodies (capture molecules) were displayed on the cell surface. 2) each cell was allowed to secrete biomolecules (antibodies in this study) for short period, which were promptly captured by the capture molecule in the vicinity of each cell. 3) each cell was labeled with fluorescence-labeled secondary antibodies (detection molecules) to establish sandwich FIA on the surface of individual cells.

RESULTS AND DISCUSSION:
In CS-FIA, the fluorescence intensity of each cell was found to correlate well with the amount of sandwiched antibodies (femtogram range). We could isolate hybridomas with higher productivity from a huge number of hybridomas within short period. CS-FIA is an effective method for single cell-based establishment of most efficient secreting cells for various biomolecules.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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CASE STUDIES IN TAILORING OF PRODUCT QUALITY ATTRIBUTES BY PROCESS DESIGN

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KEY WORDS:
PRODUCT QUALITY / RATIONAL PROCESS DESIGN / ADCC / GLYCOSYLATION

BACKGROUND AND NOVELTY:
Product quality is traditionally defined by the production process. However, this dogma is revised not only for new biological molecules in order to improve efficacy, reduce side effects, access new patient populations and the like but also for transfer of processes into new facilities and for biosimilar development. Many of the desired product properties are influenced by posttranslational modifications with impact on biological activity, immunogenicity, half life or stability. Product quality attributes have been successfully modified during cell line and upstream process development by rational selection of host cells, process parameters and media components.

EXPERIMENTAL APPROACH:
Host cell lines, process parameters and different cell culture media and supplements were selected rationally by their potential to impact product quality. Experimental verification of relevance to modulate product quality was followed by an optimization to define the best set-points. Further fine tuning of these parameters was used to improve process robustness. All experiments were carried out in controlled bioreactors including a mini bioreactor system for screening purpose.

RESULTS AND DISCUSSION:
Critical product quality attributes like antibody dependent cellular cytotoxicity (ADCC), antigen binding affinity, glycosylation, and charge heterogeneity were optimized during process development to meet a previously defined target range. Some process levers were suitable to modify a single product quality attribute, like ADCC for example. Different cell culture media compositions have shown to drive the glycosylation profile into a certain direction. In another study, antigen binding affinity and acidic peak group content were optimized in an interrelated manner enabling us to meet the previously defined target range.

MICRORNAS: ENGINEERING TOOLS TO ENHANCE CHO CELL PERFORMANCE

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KEY WORDS:
CHO / MICRORNA / GROWTH / PRODUCTIVITY / OMICS

BACKGROUND AND NOVELTY:
MicroRNAs are small non-coding RNA molecules (~ 22 nucleotides) that regulate gene expression at the post-transcriptional level. MiRNAs form a complex level of cellular regulation, for instance a single microRNA has the potential to target hundreds of mRNAs and indeed each transcript can be targeted by multiple miRNAs. The recent release of the Chinese hamster ovary (CHO) genome sequence and the addition to miRBase of over 200 mature CHO miRNA have demonstrated that miRNA in CHO are highly conserved. Previous studies in CHO cells have associated the expression of miRNAs with industrially relevant phenotypes such as growth rate, cell specific productivity and demonstrated their sensitivity to expressional modifications when exposed to environmental shifts. MicroRNA prediction algorithms are useful tools offering the researcher a means of generating lists of potential mRNA targets based on certain miRNA:mRNA binding criterion (3'Untranslated region, species conservation). However, given the short size of the microRNA "seed" region (6-8nt) and the relaxed binding criteria, the list of potential targets generated tend to be in the 100s including a high degree of false positives and negatives. Integrated "omic" profiling allows the identification of the global molecular shifts across three platforms (miRNA, mRNA and protein) thus enabling, through cross over with prediction algorithms, the identification of true microRNA targets.

EXPERIMENTAL APPROACH:
miRNA, Trancriptomic and Proteomic analysis was carried out on CHO cells with varied GRs.

RESULTS AND DISCUSSION:
The evidence presented in this study indicates that biological processes such as mRNA processing and protein synthesis are correlated with growth rate in CHO cells. We utilised the expression data in conjunction with in-silico tools to identify potential miRNA-mediated regulation of mRNA/proteins involved in CHO cell growth rate. These data have allowed us to prioritise candidates for cell engineering and/or biomarkers relevant to industrial cell culture.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
The primary work presented here was published recently: Integrated miRNA, mRNA and protein expression analysis reveals the role of post-transcriptional regulation in controlling CHO cell growth rate.
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BMC Genomics. 2012 Nov 21;13(1):656.

REF-C027

CHO HARVEST OF A LARGE SCALE SINGLE USE BIOREACTOR USING THE UNIFUGE® SINGLE USE DISPOSABLE CENTRIFUGE

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KEY WORDS:
DISPOSABLE CENTRIFUGE / SINGLE USE CENTRIFUGE / UNIFUGE / CELL HARVESTING IN SU CENTRIFUGE / CENTRIFUGE

BACKGROUND AND NOVELTY:
First Single Use Disposable Centrifuge for Harvesting

EXPERIMENTAL APPROACH:
Abstract: Production Of Chinese Hamster Ovary Proteins can be collected by Single Use centrifuge methods for harvest.

The centrifuge is very efficient from several perspectives. First the high cell density and protein rich solution can be separated from the SUB broth with the elimination of cells and debris by using a single use centrifuge. At the same time, the process is gentle on the solubilized proteins.

RESULTS AND DISCUSSION:
1) +99% life intact cell harvesting
2) no cell disruption
In Conclusion
The UniFuge is an effective tool for harvesting and clarification of CHO enriched protein titer broth. The feed flow rate of 4 lpm allows it to be used in large scale SUB production. The low shear inlet and non shear discharge mechanism allows the elimination of cells and debris as seen in the NTU measurements. In addition, The Unifuge is an effective apparatus in removing host cell proteins.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
David Richardson holds a B.A. in Biology from University of California San Diego and brings 21 years of experience in the Biopharmaceutical Industry. While an undergraduate, David was a member of a team conducting leukemia research and was published in Lancet. He started his career in 1990 with B. Braun Biotech ,and joined the original CARR Separations in 1995. He has worked in the centrifugation processing side of the business for 15 years- Including Westfalia Separator

OTHER INFORMATION
Pneumatic Scale Angelus is an exhibitor and shows continuous flow centrifuges for animal cell separation during ESACT Congress. The poster shows some results of customer test runs with the machine we will have on our booth.

REF-C028

ENGINEERING CHARACTERIZATION AND CELL CULTURE APPLICATION EXAMPLES FOR TWO DIMENSIONAL ORBITALLY SHAKEN SINGLE-USE BAGS

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KEY WORDS:
ORBITAL SHAKEN / CELL CULTURE / SINGLE-USE / COMPUTATIONAL FLUID DYNAMICS, CFD / SCALE-UP

BACKGROUND AND NOVELTY:
The acceptance and usage of orbitally shaking incubators for cell culture applications has increased during the last decade. Different orbital shaken systems from milliliter (e.g. TubeSpin) to liter (e.g. shake flasks) scale are available. Recent investigations have shown that 2D pillow-like single-use bags can be used successfully with orbital motion (INFORS HT, Multitron with ShakerBag Option). The aim of the investigations is the definition of scale -up criteria for non-similar geometries in the first place.

EXPERIMENTAL APPROACH:
However, data for engineering and fluid flow characteristics of the 2D pillow-like single-use bags with orbital movement are limited and thus, engineering characterization (mass transfer, mixing time) and numerical investigations of the fluid flow (Computational Fluid Dynamics, CFD) was carried out for the most used cultivations systems (TubeSpin, shake flask, orbitally shaken 2D pillow-like single-use bags). For the numerical investigations OpenFOAM (free and open-source software code) was used.

RESULTS AND DISCUSSION:
The mixing time and the oxygen mass transfer (kLa) were in a very good range (kLa > 20 h-1, mixing time < 30 s) for cell culture and even better than in established systems. The data of experimental research could be linked to results from CFD simulations. Additionally, CFD enables a deeper insight into the fluid flow behavior (e.g. volumetric power input, local distribution of power input, surface area), which can be used for scale-up.The successful definition of scale-up parameters is shown with application examples including the mass propagation of plant suspension cells genetically modified Nicotiana tabacum BY-2) and the mass propagation and protein formation (SEAP) of insect cells (Sf-9) in TubeSpins, shake flasks and 2D bags.

EXPANSION OF MESENCHYMAL ADIPOSE-TISSUE DERIVED STEM CELLS IN A STIRRED SINGLE-USE BIOREACTOR UNDER LOW-SERUM CONDITIONS

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KEY WORDS:
HUMAN MESENCHYMAL STEM CELLS / HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS / SCALE-UP / MICROCARRIER-SUPPORTED CULTIVATION

BACKGROUND AND NOVELTY:
The need for human mesenchymal stem cells (hMSCs) has increased enormously in recent years due to their important therapeutic potential. Efficient cell expansion is essential to providing clinically relevant numbers of cells/doses. Such cell quantities can be manufactured by means of scalable microcarrier (MC)-supported cultivations in a stirred single-use bioreactor.

EXPERIMENTAL APPROACH:
Preliminary tests in disposable-spinners (100 mL culture volume, Corning) were used to determine two suitable media and MC-types for serum reduced expansions (5 %) of human adipose tissue-derived stem cells (hADSCs; passage 2, Lonza). Using such optimized media-MC-combinations, hADSCs expanded 30 to 40-fold, which compares well with expansion rates in planar culture. Based on CFD-simulations and suspension analyses in spinners [1], optimal operating parameters were determined in a BIOSTAT UniVessel SU 2L (2 L culture volume, Sartorius Stedim).

RESULTS AND DISCUSSION:
In subsequent batch tests with the BIOSTAT UniVessel SU 2L, more than 4 • 10E8 cells with a cell viability exceeding 95 % were harvested. Flow cytometry tests demonstrated typical marker profiles following cell expansion and harvest. At present, feed strategies are being developed to further increase expansion rates to 50 - 60-fold, thus doubling yields. In summary, the foundations for successfully expanding therapeutic stem cells in truly scalable systems have been laid. Thus, a basis for a successful scale-up to pilot-scale has been provided.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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MULTI-ORGAN-CHIP - HANDLING, AUTOMATIZATION AND SUBSTANCE TESTING

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KEY WORDS:
MULTI-ORGAN-CHIP / SUBSTANCE TESTING / HIGH-TROUGHPUT / AUTOMATIZATION

BACKGROUND AND NOVELTY:
Due to the development of a Multi-Organ-Chip (MOC) by the TU Berlin's Department of Medical Biotechnology, new approaches are required to test substances in high throughput in-vitro processes. Our robot allows the user to run standard OECD substance test protocols fully automated over any given time period.

EXPERIMENTAL APPROACH:
The group develops a robotic unit for the automated handling of Multi-Organ-Chips. This system enables the user to test any substance over a time period from days up to weeks. This experimental process requires only a minimal amount of manpower to generate toxicity testing data. The MOCs can be processed with customized protocols created by the user.

RESULTS AND DISCUSSION:
The robot has the capability to carry at least 8 MOCs in its tempered operating platform. It consists of two autonomous arms to process the chips. One has a pippeting unit for maintenance and sampling, which allows the automated generation of test samples and media change at any given time point during the experiment. While the second arm carries the optical unit for fluorescence and phase-contrast pictures. The camera system can also be used for PIV measurements to determine the flow rate within the MOCs and to regulate their blood flow. All operations are controlled by a proprietary control system which logs every event in a discrete protocol. The complete robot is covered by a laminar flood shell to guarantee sterile handling at any given time point. Since the system also carries tempered tanks for media supply and sample storage.

REF-C031

SERUM-FREE SUSPENSION CULTURE OF HUMAN CELL LINES: SK-HEP-1 E HKB-11

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KEY WORDS:
SERUM-FREE MEDIA / HUMAN CELL LINES / SUSPENSION CULTURE / SERUM-FREE ADAPTATION

BACKGROUND AND NOVELTY:
The majority of recombinant biopharmaceutical products are expressed in mammalian cells due to the correct post translational modifications as well as protein folding in an authentic way, thus generating a protein with suitable therapeutic quality. Human cell lines have attracted great interest since they are capable of producing glycosylated proteins in a more similar way to native human proteins, reducing the potential for immune responses against non-human epitopes. The human cell lines Hek293, Per.C6, HKB-11, SK-Hep-1 have attracted interest due to their properties.

EXPERIMENTAL APPROACH:
The human cell lines HKB-11, SK-Hep-1 were obtained from the American Type Culture Collection and maintained at -194ºC. Four commercial formulations were analyzed for the establishment of fetal bovine serum (FBS) free culture: Free Style 293 Expression Medium, CD 293 AGT, 293 SFM II (Invitrogen), CDM4-CHO (HyClone). The cultures were grown in T flasks kept at 37ºC, 5% CO2. Whereas the lines were originally grown in DMEM +10% FBS, a procedure of adaptation to new media had to be performed. In each passage, the portion containing FBS was decreased by 25% and the culture remained at this concentration for 2-3 passages. The best medium was selected for further suspension adaptation in spinner and erlenmeyer flasks.

RESULTS AND DISCUSSION:
The population doubling (PD) for human cell lines SK-Hep-1, HKB-11 in DMEM 10% FBS were 2,2; 3,0, respectively. Through adaptation, only the cell line SK-Hep-1 in CDM4 (PD=1,46) and SFMII (PD=1,68) culture and the cell line HKB-11 in CDM4 (PD=1,8) and Free Style 293 (PD=1,8) culture survived through the following serum free passages. The specific growth rate constant (μmax) and maximum cellular density (Xmax) for SK-Hep-1 and HKB-11 in CDM4 spinner suspension culture were 0,031 and 0,024 h-1 and 2,14x106 and 3,92x106 cel/mL, respectively. In CDM4 erlenmeyer suspension culture, the μmax and Xmax for SK-Hep-1 and HKB-11 were 0,010 and 0,028 h-1 and 1,37x106 and 8,01x106 cel/mL, respectively.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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Centro de Hemoterapia de Ribeirão Preto (Regional Blood Center of Ribeirão Preto) Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2012/02109-7).

REF-C032

SCREENFLEX - TAILORED EXPRESSION CELL LINES

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KEY WORDS:
PROTEIN EXPRESSION / TARGETING / MODULATION / PRODUCTION / DRUG DISCOVERY

BACKGROUND AND NOVELTY:
The production of pharmaceutical proteins requires high-level expression, whereas other proteins should be expressed in a more balanced matter. Our SCREENflex technology has been generated to establish cell lines with tailored expression levels for virtually any desired purpose – within 4 weeks.

EXPERIMENTAL APPROACH:
SCREENflex is a combination of pre-selected CHO or HEK293 master cell lines and special expression vectors. The master cell lines are tagged by a reporter cassette. In a highly specific molecular "cut and paste" step the reporter cassette will be exchanged for expression vectors carrying the gene of interest – resulting in the final tailored expression cell lines.

We followed the theory, that the use of a certain reporter causes biased vector integration. These integration sites may support the expression of proteins similar to the reporter but not necessarily other protein classes. Recently we described the application of SCREENflex to generate cell lines for drug discovery. A receptor served as tagging reporter and was successfully exchanged for several GPCRs. Now we could show, that also the level of reporter expression of individual master cell lines can be transferred to the final expression cell lines. We applied low, medium and high level expressing master cell lines tagged with either an intracellular or a membrane-spanning reporter. The expression of the final daughter cell lines followed the level of the master cell lines if the new gene belonged to the same protein class as the tagging gene.
Further we were able to fine-tune the expression level of target genes by the use of different promoters. Therefore SCREENflex offers two levels of expression modulation: I. the genomic site per se and II. genetic elements to drive transgene expression.

RESULTS AND DISCUSSION:
The results show, that SCREENflex is an ideal system for optimal expression modulation of a desired transgene.

SEED TRAIN OPTIMIZATION FOR SUSPENSION CELL CULTURE

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KEY WORDS:
SEED TRAIN / OPTIMIZATION / MODELLING / SPACE-TIME-YIELD / SUSPENSION

BACKGROUND AND NOVELTY:
The production of biopharmaceuticals for diagnostic and therapeutic applications based on suspension cell culture in bioreactor scales from a few hundred litres up to 20 m3 is state of the art. The generation of an adequate number of cells for the inoculation of a production bioreactor is time- and cost-intensive. From volumes used for cell thawing or cell line maintenance the cell number has to be increased while usually passaging into larger cultivation systems. Examples are T-flasks, roller bottles or shake flasks, small scale bioreactor systems and subsequently larger bioreactors. More and more, disposable technology is applied. In order to reduce the number of passages within the seed train also systems are used which are inoculated at a partly filled state and culture volume is increased afterwards by medium addition. However, the seed train lasts for a significant period of time and generates corresponding costs, e.g. from a 5 mL-scale until inoculation of a 3 m3-scale in the range of 21 days. Simultaneously, the seed train offers space for optimization, for example via the choice of the optimal point of time for passaging from one scale into the larger one or via the choice of inoculation density and culture volume at inoculation.

EXPERIMENTAL APPROACH:
A suspension cell seed train has been displayed in the program Matlab. Cell growth, cell death, substrate uptake and metabolite production can be adapted via a model for different cell lines as well as the corresponding medium concentrations.

RESULTS AND DISCUSSION:
The concept and the tool for the seed train optimization will be presented as well as results based on data of a CHO cell line which show an optimized seed train in comparison to a standard seed train using fixed time intervals. A second poster will present the application to a lab scale seed train as well as experimental realisation and verification ("Model-based design of the first steps of a seed train for cell culture processes").

DATA INTEGRATION METHODOLOGY THAT COUPLES NOVEL BIOREACTOR MONITORING TOOLS, AUTOMATED SAMPLING, AND APPLIED MATHEMATICS TO REDEFINE BIOPRODUCTION PROCESSES

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KEY WORDS:
BIOREACTOR MONITORING TOOLS / AUTOMATED SAMPLING / DATA INTEGRATION / APPLIED MATHEMATICS

BACKGROUND AND NOVELTY:
Cell physiology dynamically affects the nutrient requirements of a culture. It is critical to obtain data over appropriate time intervals to assess the impact of process conditions on the cell population. By optimizing bioreactor operation, feed strategies and media composition, we can limit the number of experiments to obtain the empirical data sets.

EXPERIMENTAL APPROACH:
For this poster, we present an emerging process-development methodology that is based on applying novel and existing bioreactor monitoring technologies, coupled with applied mathematics, to bioreactor processes. This approach employs tools like dielectric spectroscopy, aseptic autosamplers, and cell-based bioreactor models. We will illustrate how information gained from these tools can be coupled through utilization of the proper data integration and applied mathematics techniques.

RESULTS AND DISCUSSION:
The knowledge gained using this improved process development methodology also supports a less-invasive monitoring and feedback system, and can be implemented using a customized bioreactor control code.

REF-C035

MAMMALIAN SYSTEMS BIOTECHNOLOGY FOR CHARACTERIZING CHO CELL AND HESC CULTURES

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KEY WORDS:
SYSTEMS BIOTECHNOLOGY / CHO CELL / HESC / GENOME-SCALE MODEL / METABOLOMICS

BACKGROUND AND NOVELTY:
The increasing demand for recombinant therapeutic proteins highlights the need to constantly improve the efficiency and yield of these biopharmaceutical products from mammalian cells, which is fully achievable only through proper understanding of cellular functioning. Toward this end, we recently developed "mammalian systems biotechnology" framework where omics data-driven and hypothetical model-driven approaches are integrated to study the growth characteristics of mammalian cells from a systems perspective as well as identify key cellular targets for enhancing the culture performance.

EXPERIMENTAL APPROACH:
As one of case studies, the current work exploited a combined metabolomics and in silico modeling approach to gain a deeper insight into the cellular mechanisms of Chinese hamster ovary (CHO) fed-batch cultures.

RESULTS AND DISCUSSION:
Initially, extracellular and intracellular metabolite profiling analysis shortlisted key metabolites associated with cell growth limitation within the energy, glutathione, and glycerophospholipid pathways that have distinct changes at the exponentialstationary transition phase of the cultures. Subsequent in silico modeling of CHO cells characterized internal metabolic behaviors attaining physiological changes during growth and non-growth phases, thereby allowing us to explore relevant pathways to growth limitation and identify major growth-limiting factors including the oxidative stress and depletion of lipid metabolites. Such key information on growth-related mechanisms derived from the current approach can potentially guide the development of new strategies to enhance CHO culture performance. As another case study, similar approaches can be applied to characterize the phenotypic.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
This work was supported by the National University of Singapore, the Biomedical Research Council of A*STAR (Agency for Science, Technology and Research), the National Research Foundation of Singapore (NRFCRP5-2009-03), and a grant from the Next-Generation BioGreen 21 Program(SSAC, No. PJ008184), Rural Development Administration, Republic of Korea.

REF-C036

OPTIMIZING THE PRODUCTIVITY OF A RECOMBINANT MONOCLONAL ANTIBODY-PRODUCING CHO CELL LINE: DEVELOPMENT OF REAL-TIME ANALYTICAL TECHNIQUES FOR MONITORING BIOPROCESS AND IMPLEMENTATION OF A CONTROL STRATEGY

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KEY WORDS:
NEAR-INFRARED SPECTROSCOPY / MAMMALIAN CELL CULTURE / OPTIMIZATION OF PROTEIN PRODUCTION / IN-LINE MONITORING / ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

BACKGROUND AND NOVELTY:
Complex biopharmaceutical products are industrially produced by genetically modified mammalian cells grown in bioreactors. At industrial level, real-time monitoring of bioreactors is limited to a few parameters and control is often merely based on empirical knowledge. Since near-infrared spectroscopy (NIRS) offers numerous assets (real-time, in situ and multi-analyte quantification), this analytical technique has been largely investigated in the last decade. NIRS has been applied to monitor main nutriments (glucose and glutamine) and inhibitors (lactate and ammonium ions) of CHO cells. The NIRS monitoring is planned to be extended to the amino acids limiting or inhibiting the protein production. The real-time and in situ monitoring progresses will allow the design of a dynamic feeding strategy that meets the real needs of the cells grown in the bioreactor.

EXPERIMENTAL APPROACH:
The analytes that limit the protein production must be identified and included in the calibration model enabling us to monitor the bioprocess. With that purpose in mind, models of increasing complexity are developed in order to assess the ability of the NIRS to monitor components present at low concentrations, below 1 mM. On basis of these developments, a control strategy of the cell culture medium composition will be designed.

RESULTS AND DISCUSSION:
The effort of calibration of the NIRS has confirmed the possibility of monitoring four analytes in synthetic solutions containing mammalian cells and matrix effects associated to the cell culture medium. Encouragingly the limits of quantification of the analytes have been estimated inferior to the limiting concentrations levels reported in the literature. However the solutions that have been measured so far are by far less complex than the bioprocess intended to be monitored in fine. The model designed to monitor the bioprocess will be developed with calibration spectra collected in solutions with varying concentrations of both the desired analytes and all potentially interfering compounds.

DYNAMIC CYCLIN PROFILING AS A TOOL TO SEGREGATE THE CELL CYCLE

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KEY WORDS:
CYCLINS / CELL CYCLE / GS-NS0

BACKGROUND AND NOVELTY:
Mammalian cells growth, productivity and cell death are highly regulated and coordinated processes. The cell cycle is at the centre of control and has the potential to aid in determining optimization strategies towards improving productivity [1]. Specifically, cell productivity is cell cycle, cell-line and promoter dependant [2]. The cyclins -key regulators- activate their partner cyclin-dependent kinases (CDKs) and target specific proteins driving the cell cycle. To our knowledge, there is no information on cyclin phase-dependent expression profiles of industrial relevant mammalian cell lines. We use the cyclins' profiles as a tool to identify and quantify the landmarks of the cell cycle and implement it in a modelling approach to describe the cell system.

EXPERIMENTAL APPROACH:
Cyclins' expression (cyclin E - G1 class and cyclin B - G2 class) was studied in GS-NS0 batch cultures by flow cytometry. The cells were cultured under both perturbed (cell arrest) and unperturbed growth. The static profiles were obtained by direct cyclin staining and the dynamic profiles were reconstructed by either a) combining the timings from proliferation assays with the static profiles, b) tracking a partially synchronized population, or c) by the simultaneous staining for proliferation and cyclin expression.

RESULTS AND DISCUSSION:
The different approaches for deriving the dynamic cyclins' profiles provide a versatile experimental toolbox for the cell cycle characterisation. Cyclins can be used as modelling distributed variables and be experimentally validated (quantitatively), avoiding the use of weakly supported variables (e.g. age, volume). Both cyclins showed a clear cell cycle phase specific pattern (cyclin E 10% higher at G1 and cyclin B 40% higher at G2/M). The observed patterns and timings provide a blueprint of the cell line's cell cycle, which are used for cell cycle modelling. The development of these models will aid the systematic study of the cell culture system, the improvement of productivity and product quality.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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NIR-SPECTROSCOPY FOR BIOPROCESS MONITORING & CONTROL

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KEY WORDS:
NEAR INFRARED SPECTROSCOPY / FERMENTATION / CULTIVATION / BIOPROCESS / BIOPROCESS MONITORING AND CONTROL

BACKGROUND AND NOVELTY:
Monitoring the critical process parameters during biotechnological cell cultivations is of high importance for maintaining a high efficiency and quality of a bioprocess. Optimizing and ensuring a high efficiency of a bioprocess requires monitoring and controlling of parameters such as cell metabolites like glucose consumption and the production of lactate. This can be achieved by different offline analyses. In this case one has to accept that the offline analysis has a long reaction time and for this reason only offers a limited ability for process control. Much better suited technologies can be used directly in the reactor. They will give a direct real-time feedback of the process parameters which will lead to a full process control and ease the process understanding. NIR spectroscopy enables multiparametric, non-invasive measurements of substrates, metabolites, products, cell parameters and process trajectories.

EXPERIMENTAL APPROACH:
This work shows the results of the investigation of the usage of a specially designed NIR system which can be connected to a bioprocess through a standard Ingold port by being completely free of optical fibers and therefore being able to stand a CIP/ SIP process. The investigated process is a CHO-K1 cultivation which has been used to show the ability of fiber free NIR spectroscopy to monitor and visualize quantitative parameters such as nutrients, metabolites or cell parameters and use qualitative parameters to monitor the process trace.

RESULTS AND DISCUSSION:
It has been approved that the use of this specialized NIR process system enables the inline monitoring of the cell count and viability due to the construction with a free beam optics as the most important qualitative parameters which can be measured with this approach. The use of a principal component analysis of the spectral data enables a sophisticated process monitoring over time. Using this time depending process fingerprint over time one has a very good tool to compare individual batches assuring a constant quality.

REF-C039

SYSTEMS APPROACH FOR MEDIA FORMULATION AND OPTIMIZATION

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KEY WORDS:
MEDIA FORMULATION / DOE / CHO / OPTIMIZATION / BIOSILIMAR

BACKGROUND AND NOVELTY:
Traditional methods of media development are time and resource intensive. Recently DOE (Design of Experiment) has become a useful and convenient tool for designing media formulation and optimizing the corresponding processes along with the need of a chemically defined media. However, the media development and optimization still remains as a bottlenecking point in biosilimar or bio-therapeutics development, and media compositions are main manipulators to meet product quality specification. This process involves lots of heuristics, and trial and error.

EXPERIMENTAL APPROACH:
In this study, the design of media formulation is formulated as a multi-variable optimization problem while maximizing productivity and meeting product quality specifications. The new mathematical framework was tested with a DG44 CHO cells. The DG44 CHO cells being an expressing cell line, the titer (amount of IgG production), VCD (cells/ml), glycosylation and the viability (%) were the criteria for deciding the optimum concentration of each of the components to be tested.

RESULTS AND DISCUSSION:
This strategy allowed saving a lot of time and resources and obtaining an extensive data set, which provided the information on all media components on an individual level and in association and eventually reduce the development time for any bio-therapeutics. However, there still remain some challenges, i.e. if the same regression models can be used for other cell-lines, how many baseline datasets will provide reliable results for media formulation, and how one can adapt the new media development technology for biosimilar and bio-therapeutics development. In the presentation, achievement and current challenges will be addressed.

REF-C040

EXPRESSION OF RECOMBINANT IGA ANTIBODIES AND IN-DEPTH CELL LINE CHARACTERIZATION

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KEY WORDS:
RECOMBINANT IGA / CHO / IG CLASS SWITCH / EXPRESSION BOTTLENECKS

BACKGROUND AND NOVELTY:
Immunoglobulin A (IgA) mediates a key role in mucosal immunity and is a promising novel immunotherapeutic candidate. However, difficulties in obtaining enough material often hamper in vivo explorations. We have previously generated recombinant Chinese hamster ovary (CHO) cell lines which expressed two different HIV-1 antibodies, 3D6 and 4B3, as IgA1 (Reinhart et al. 2012). Substantial differences in specific productivities among the established cell lines encouraged us to perform an in-depth characterization to unravel the underlying cause(s).

EXPERIMENTAL APPROACH:
The two monoclonal antibodies 3D6 and 4B3 have originally been isolated as IgG1 isotype from seroconverted HIV-1 patients and bind to the principal immunodominant domain of gp41. Both mAbs were isotype switched to IgA1 and subsequently expressed as dIgA in CHO cells under serum-free conditions (Reinhart et al. 2012). The generated cell lines were extensively characterized according to growth rate, specific productivity, gene copy number (GCN) and mRNA levels. Furthermore, immunofluorescence microscopy, flow cytometry and Western blotting of intra- and extracellularly expressed protein was employed to examine protein folding/assembly and potential ER stress responses.

RESULTS AND DISCUSSION:
Differences in specific productivity of the cell lines could not adequately be explained by qPCR data of GCN and mRNA levels. Via flow cytometry intracellular antibody light and heavy chains were spotted but without any major expression differences. However, flow cytometry additionally highlighted a potential ER stress response in one of the cell lines by the over expression of the KDEL signal being specific for ER chaperones. Using immunofluorescence microscopy and Western blotting bottlenecks in polypeptide assembly could be identified which may have been the trigger for the ER stress response.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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OTHER INFORMATION
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ASSESSING THE EXOMETABOLOME AND FLUXOME OF CHO CELLS FOR PREDICTIVE BIOPROCESS IMPROVEMENT

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KEY WORDS:
CHO CELLS / PRODUCTIVITY OPTIMIZATION / METABOLIC FLUX ANALYSIS / 1H-NMR METABOLITE PROFILING

BACKGROUND AND NOVELTY:

CHO cells are preferred hosts for the production of recombinant biopharmaceuticals. Efforts to optimize these bioprocesses have mostly relied on empirical experience, but our knowledge of cellular performance in culture is still modest. More recently, systems biotechnology tools have started to be used to uncover the molecular traits of optimal growth and protein production. In particular, a comprehensive investigation of metabolic network operation could uncover important signatures that impact specific monoclonal antibody (mAb) yields.

EXPERIMENTAL APPROACH:

In this work, a 1H NMR protocol was implemented to quantify supernatant compounds throughout culture time in CHO clones expressing variable amounts of a mAb under standard and butyrate treated conditions. Exometabolomic data was integrated through metabolic flux analysis (MFA) allowing the estimation of cellular fluxomes. A hybrid MFA approach based on projection to latent structures was then applied on the generated data to assess metabolic signatures associated with hyperproductivity. Finally, culture supplementation strategies were designed based on this analysis and experimentally validated.

RESULTS AND DISCUSSION:

We were able to accurately quantify 39 supernatant metabolites along culture time, including by products previously unmeasured in CHO cells, allowing the resolution of a comprehensive network of 117 intracellular and transport reactions. A comparison of cellular metabolism at different productive states showed meaningful alterations of central metabolic activity at different metabolic phases (growth vs stationary) as well as resulting from butyrate treatment. Hybrid MFA analysis showed marked correlation strengths between individual fluxes and productivity, based on which a rational medium supplement was designed and experimentally validated to further improve mAb specific yields. This work demonstrates how systems biotechnology approaches improve our understanding of cell behaviour and can be translated in predictive bioprocess improvement.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

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SPEEDING UP PROCESS DEVELOPMENT WITH AN AUTOMATED MICROTITER PLATE BASED SYSTEM FOR SUSPENSION CELL CULTURE

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KEY WORDS:
CELL CULTURE / AUTOMATION / PLATE / SHAKEN / FED BATCH

BACKGROUND AND NOVELTY:

Process automation provides the appropriate tools to address the following key points:

Increasing experimental throughput --> enable full factorial design of experiments
Increasing process information --> improve process understanding
Automate repetitive manual work --> gain efficiency, focus on high value tasks

EXPERIMENTAL APPROACH:

We developed an automated, multiwell plate based screening system for cell culture processes. The system is setup to be generic and can utilize multiwell plates of different configurations as bioreactors (6 to 48 wells per plate). The screening system is based on off-the-shelf commercial laboratory automation equipment. It is fully automated and handles plate transport, feeding and seeding of cells, daily sampling and preparation of metabolite assays.

RESULTS AND DISCUSSION:

1. Scale-up prediction

The comparability of results obtained with the multiwell plate based system and bioreactors had to be verified. It could be shown that 6 well plates were predictive for a scale-up to a 1,000 L stirred tank reactor (scale factor 1:200,000).

2. Media screening

An increase in viable cell density and product titer of about 20% in comparison to the reference process was achieved.

Several 2 L bioreactor runs using these optimized parameters later confirmed these predicted results.. Clone screening

The standard manual protocol was compared to an automated protocol using 96 clones. The automated approach screened the clones in duplicates using shaken 24 well plates and fed-batch mode. Key metabolites were measured daily and the glycosylation pattern was analyzed at harvest for all clones.

All duplicates showed reproducible performance and the overall top producer was initially identified only by the automated process but later confirmed in the manual process. The automation enabled the screening of a larger number of clones using a process very similar to the final manufacturing (platform) process while also providing metabolic profiles.

REF-C044

HOW TO ASSESS CHEMICALLY DEFINED MEDIA AND FEEDS FROM 7 SUPPLIERS ON CHO-S CELLS PRODUCING MAB

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KEY WORDS:
CHO CELLS / MEDIUM / FEED / MAB / SCREENING

BACKGROUND AND NOVELTY:
Clone screening and selection is more and more performed directly in medium and feed platforms in order to select the best performers and reduce process development timelines. Merck Biodevelopment implemented a complete protocol to adapt, assess and choose the best medium and feed platforms on CHO-S cells.

EXPERIMENTAL APPROACH:
12 media and 16 feeds from 7 suppliers (Life Technologies, SAFC, GE-PAA, BD, Irvine, Lonza and Thermo-Hyclone) in addition to our internal platform were assessed on 3 CHO-S cells producing mAb. Cells were adapted with a 2-phase approach in order to reduce growth issues. When regular cell growth was observed, batch and fed-batch experiments were launched resulting in 52 spin tubes. Glucose and glutamate concentrations in addition to pH and osmolality of each medium and feed were also compared.

RESULTS AND DISCUSSION:
While pH is quite similar between all media and feeds, osmolality values are widespread. Our study highlighted robust platforms, cell growth enhancer or specific productivity dedicated platforms. Results comparison between batch and fed-batch allowed to separate the feed impact from the medium itself. This approach brought out the best performers with, in somes cases a 4 fold productivity increase. Finally practical aspect was also compared between platforms to complete our assessment and give recommendations.

REF-C045

PPC - A NOVEL POLYCATIONIC REAGENT FOR TRANSIENT TRANSFECTION OF MAMMALIAN CELLS

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KEY WORDS:
TRANSIENT GENE EXPRESSION / PEI / MAMMALIAN CELLS / TRANSIENT TRANSFECTION / PPC

BACKGROUND AND NOVELTY:
For rapid recombinant protein production in small to medium size batches, transient transfection of mammalian cells is still the method of choice in biotechnology. However, the transfection reagent most notably remains a cost intensive bottleneck. As a consequence, there is a strong and continued interest for novel transfection reagents that exhibit higher DNA-transfer efficiencies, with lower toxicity, than the widely used polyethylenimine (PEI), all without the high cost associated with lipofectamines.

EXPERIMENTAL APPROACH:
The task at hand for emp Biotech was the design and synthesis of various functionalized polycationic moieties that can mediate between polyanionic plasmid DNA and the negatively charged cell surface, thereby facilitating uptake of DNA into the cell. Two improvements were of primary interest. For increased solubility hydrophilic functional groups were integrated into the polymer structure and to reach lower cytotoxicity cleavable ester groups allow rapid intracellular degradation. Chemically synthesized candidates were provided to InVivo Biotech.

RESULTS AND DISCUSSION:
Cytotoxicity was tested over a broad range of concentrations. Results demonstrate several novel synthetic polymers exhibiting transfection efficiencies even higher than common PEIs after optimized ratios of DNA-to-polymer were applied. Further experiments using secreted alkaline phosphatase (SEAP) as a reporter for transient protein expression have indicated that these substances are at least equal to PEI, and notably one which actually enables higher expression levels when compared to transient transfections using commercially available PEIs. The synthesis of this reagent was optimized and standardized for further improvement of productivity. Therefore the polymer as transfection reagent was characterized by different analysis techniques. Furthermore the stability of the reagent concerning pH and time were tested in long-term studies and protocols for adherent and suspension cultures were established for various cell lines.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

OTHER INFORMATION
In conclusion, our results using HEK 293, CHO and CAP-T cells demonstrate the development of a promising new alternative transfection reagent for transient protein expression.

REF-C046

CELL CYCLE AND APOPTOSIS: A MAP FOR THE GS-NS0 CELL LINE AT THE GENETIC LEVEL AND THE LINK TO ENVIRONMENTAL STRESSES

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KEY WORDS:
APOPTOTIC GENES / CELL CYCLE / MONOCLONAL ANTIBODY / GS-NS0

BACKGROUND AND NOVELTY:
Environmental stresses affect the production of monoclonal antibodies (MAbs). These stresses have an impact at the genetic level, disrupting the cell cycle, triggering apoptosis, resulting in a low MAb titre. Control of the cell cycle and apoptosis has been investigated in order to achieve higher MAb titres by either apoptosis inhibition via bcl-2 overexpression or cell arrest at G1/G0 by p21 transfection – approaches that have not always been successful [1-3]. Consequently, a systematic insight of the dynamic relation between metabolic stress and the genetic regulation of the cell cycle and apoptosis is still needed. To this end, our aim is to establish a novel and wider map of the interplay between the cell cycle and apoptosis starting at the genetic level and the interlink with environment and metabolic stresses.

EXPERIMENTAL APPROACH:
Batch cultures of GS-NS0 producing a cB72.3 MAb were performed. Cell density and viability were quantified. Extracellular glucose, glutamate, lactate and ammonium were quantified using a Bioprofiler. Extracellular MAb was quantified via ELISA. DNA staining and Annexin V/PI assay was used to quantify the cell cycle fractions as well as the degree of apoptosis. The measurement of apoptotic (atf5, trp53bp2, casp3, casp8) and cell cycle (p21, bcl2) related genes was conducted using real-time PCR.

RESULTS AND DISCUSSION:
Our results showed a clear dynamic interaction between environmental stresses and the genetic levels. atf5 upregulation was linked to glutamate depletion, in agreement with previous results [4]. Lactate accumulation upregulated trp53bp2 whilst casp8 upregulation was associated with high cell density. casp3 showed a clear association with the increasing apoptotic population. In addition, upregulation of p21and bcl-2 showed a clear link to the increase of the G1/G0 fraction, indicating a mechanism associated with the prevention of cell death. This blueprint will aid the systematic study of the culture system towards the improvement of the MAb productivity.

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REF-C047

SYSTEMS BIOLOGY OF UNFOLDED PROTEIN RESPONSE IN RECOMBINANT CHO CELLS

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KEY WORDS:
CHO CELLS / PRODUCTIVITY / UNFOLDED PROTEIN RESPONSE (UPR) / ER STRESS / SYSTEMS BIOLOGY

BACKGROUND AND NOVELTY:
Productivity of recombinant therapeutics is a coordinated effort of multiple pathways in the cell. The protein processing pathway in endoplasmic reticulum has been the target of many cell engineering studies but with mixed results. In this work, we apply a systems biology approach encompassing experiments and computation to understand the response of this pathway in high productivity CHO cells.

EXPERIMENTAL APPROACH:
Recombinant CHO cells secreting an anti-rhesus IgG antibody are used for this study. All batch cultures in a media containing equal volumes of PF-CHO and CD-CHO were monitored for viable cell density, metabolite concentrations and IgG titers. Recombinant mRNA levels were quantified using real time PCR using beta-actin as a housekeeping gene. Primers were designed based on consensus sequences from human, mouse and rat and checked against the CHO genome database wherever available.

RESULTS AND DISCUSSION:
To understand the role of ER stress in these cells, mRNA levels of key chaperones and UPR signaling pathway proteins are profiled along multiple time points of the growth curve. Comparison of a high and low producer in batch culture revealed induction of many chaperones in the high producer early in growth. Both the integrated stress response (ISR) and Ire1-alpha signaling branches of UPR were strongly upregulated in the high producer. The time of induction correlated with that of peak productivity. Since UPR can be induced by both nutrient deprivation and unfolded proteins, further studies are being carried out to determine whether starvation of nutrients is linked to UPR upregulation. The response to ER stress modulators including tunicamycin and valproic acid is also being studied in these cells for comparison. The gene expression profiles along with the metabolite data from the different batch cultures and cell lines is used in a multiple regression model to determine factors contributing to productivity.

REF-D001

DEVELOPMENT OF SERUM-FREE MEDIA FOR HUMAN MESENCHYMAL STEM CELLS EX VIVO EXPANSIONS

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KEY WORDS:
MESENCHYMAL STEM CELL / SERUM FREE / EX VIVO EXPANSION / CELL THERAPY / REGENERATIVE MEDICINE

BACKGROUND AND NOVELTY:
Human mesenchymal stem cells (MSCs) have been shown to offer a great potential to treat stroke, spinal cord injuries, cancer and serious heart disease in many pre-clinical studies. A major challenge for this potential to become a clinical reality is to establish a safe, rapid and consistent large scale MSC expansion protocol. Fetal bovine serum (FBS) is often required to effectively expand human MSCs; however, sera pose numerous safety issues and introduce inconsistency to the expansion outcome. To address the concern of using FBS for clinical grade MSCs production, a serum-free (SF) MSCs expansion medium was developed at Irvine Scientific (IS).

EXPERIMENTAL APPROACH:
A SF medium containing optimal compositions of recombinant human proteins was developed and tested for expansions of MSCs derived from bone marrow (BM) and adipose tissue (AT). Cell surface marker expression, proliferation, morphology, immunomodulation assay and differentiation analysis were used to evaluate the medium performance in comparison with FBS-containing medium and other commercially available SF media. The performance of IS SF medium in supporting expansion of primary MSCs from biopsies and its application in 3D culture systems were also evaluated.

RESULTS AND DISCUSSION:
Both BM- and AT-derived MSCs were successfully expanded using IS SF medium. Cells grown in IS SF medium showed comparable spindle morphology and doubling times with cells grown in FBS-containing medium during expansion. They were positive for CD90, CD105 and negative for CD45. Furthermore, they effectively suppressed the proliferation of activated T cells and were capable of differentiating toward adipogenic, osteogenic and chondrogenic lineages using specific in vitro conditions. We also demonstrated IS SF medium can support growth of primary MSCs directly from biopsies. When testing its application in 3D culture systems, IS SF medium delivered FBS-comparable results. However, the 3D culture protocol needs to be further optimized to achieve desired performance.

REF-D002

A NEW CELL DETACHMENT REAGENT WHICH REPLACES TRYPSIN FOR USE IN CELL THERAPY, VACINE & BIOPHARMACEUTICAL PRODUCTION

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KEY WORDS:
CELL CULTURE / TRYPSIN / CELL DETACHMENT / HESC / ACCUTASE

BACKGROUND AND NOVELTY:
Accutase cell detachment solution is a ready to use non-mammalian replacement for all applications of trypsin that performs exceptionally well in dissociating cell-cell and cell-surface attachments, even though its protease activity is less aggressive than trypsin. In addition Accutase improves culturing cells for a variety of applications, including hESC, flow cytometry, virus production, most cell-based assays, routine cell passage and bioproduction. Previously, Accutase has been shown to yield superior results when replacing the use of trypsin in cell culture applications, namely higher viability, greater cell recovery and faster reattachment times. These features have increased the use of Accutase in stem cell, vaccine and biopharmaceutical settings, thereby requiring the development of a new grade of Accutase for these markets.

EXPERIMENTAL APPROACH:
Samples of the new formulation, AccutaseGMP, and the original Accutase formulation were sent to investigators for a comparative evaluation in their standard protocols for use with their cell lines of choice. The investigators were asked to report any differences in cell yield, viability, morphology, plating efficiency, growth characteristics after reattachment, and if possible, functional assay differences between the two formulations.

RESULTS AND DISCUSSION:
The data show that there were no differences between Accutase and AccutaseGMP with regard to cell yield, viability, morphology, plating efficiency, growth characteristics after reattachment, or functional assay differences. Cell lines tested included KhES-1, H9, hESC-LN 57001, hIPSC lines, IMR90, 253G1, 35 IPS lines, proprietary hESC cells, HeLa, CHO, Mosec, Hep G2, 4T1, HCT-116, HEK 293, HeK293T, MDCK and CHO-KI. With the recent EMEA guidelines aimed at decreasing the porcine trypsin-based AVA contamination (i.e., parvovirus and circovirus) risk of products in cell therapy, vaccines and biopharmaceuticals, AccutaseGMP provides an effective and rational alternative for these cell detachment applications.

TOWARD A SERUM-FREE, XENO-FREE CULTURE SYSTEM FOR OPTIMAL GROWTH AND EXPANSION OF HUMAN MSC SUITED TO THERAPEUTIC APPLICATIONS

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KEY WORDS:
HUMAN MESENCHYMAL STEM CELLS (HMSC) / SERUN-FREE (SF) / XENO-FREE (XF) / ANIMAL COMPONENT FREE (ACF)

BACKGROUND AND NOVELTY:
Human mesenchymal stem cells (hMSC) hold great promise as tools in regenerative medicine and cell therapy. Application of hMSC in cell therapy requires the elaboration of an appropriate serum-free (SF), xeno-free (XF) culture system in order to minimize the health risk of using xenogenic compounds, and to limit the immunological reactions in-vivo. Beside the well-known disadvantages of serum, in comparison to SF, XF culture system, serum also exhibits poor performance in the context of hMSC proliferation.

EXPERIMENTAL APPROACH:
Development of the SF, XF culture system was conducted on hMSC from a variety of sources: bone marrow (BM), adipose tissue (AT) and Wharton's jelly (WJ). The MSC Nutristem® XF culture medium was examined in combination with MSC attachment solution and recombinant trypsin dissociation solution. The performance of the MSC Nutristem® XF culture medium was evaluated based on the following parameters: proliferation rate, viability, morphology, stemness (estimated from CFU-F), tri-lineage differentiation capacities, and phenotypic surface marker profile.

RESULTS AND DISCUSSION:
Results show that the complete SF, XF culture system for hMSC that was developed, composed of MSC Nutristem® XF medium and the necessary auxiliary solutions, supported optimal expansion of hMSC from a variety of sources, and exhibited superior proliferation compared with serum-containing media. hMSC expanded in the SF, XF culture system maintain their typical fibroblast-like cell morphology and phenotypic surface marker profile of CD73, CD90, CD105, HLA-ABC (all positive), or CD34, CD45, HLA-DR (all negative). hMSC differentiated efficiently after expansion in the developed SF, XF culture system as demonstrated by differentiation into osteocytes, chondrocytes and adipocytes. The self-renewal potential was maintained as well, demonstrated by colony-forming unit fibroblasts (CFUF) assay.
To summarize, the developed SF, XF culture system enables long-term growth of multipotent hMSC suitable for therapeutic applications.

SCALABLE MICROCARRIER-BASED EXPANSION OF STEM CELLS

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KEY WORDS:
STEM CELLS / MICROCARRIER / REGULATORY-COMPLIANT / BIOREACTOR / EXPANSION

BACKGROUND AND NOVELTY:
Significant interest in Mesenchymal stem cells (MSCs) has been generated because these cells hold the potential to cure disease and are being evaluated in clinical trials. For eventual implementation of these therapies, there is a tremendous need to develop a cost-effective technology which will provide reliable, reproducible, and scalable production of adherent stem cells. Current expansion processes utilize flat-surface formats, which limit the ability for scale-up. Microcarriers represent an ideal replacement substrate for cell expansion. Their benefits include increased process control and reproducibility in computer-controlled bioreactors and reduced footprint. Microcarriers of various size ranges and densities also promote high levels of cell attachment and growth and plastic microcarriers facilitate efficient recovery of viable cells. Results presented here demonstrate that microcarriers provide a desirable platform for producing large numbers of undifferentiated MSCs.

EXPERIMENTAL APPROACH:
We used multiple microcarrier types to develop novel protocols for generating large numbers of cells in stirred tank reactors. In these studies we optimized cell attachment kinetics and efficiency to microcarriers in commercially available media formulations. MSC growth on microcarriers was evaluated and cell identity and differentiation potential was examined after cell expansion.

RESULTS AND DISCUSSION:
We have developed protocols for microcarrier-based expansion of MSCs on regulatory-compliant microcarriers. Cell densities reached on microcarriers were greater than those obtained on flatware and superior cell yields were obtained, making it possible to produce ~ 1 billion MSCs in 4 to 5 L of media. MSCs remained in an undifferentiated state and retained the ability to differentiate into adipocytes and osteocytes. Our results demonstrate the feasibility of using microcarriers as a cost-effective substrate for expansion of MCS and underscore their utility as a reliable platform for scale-up in bioreactors.

REF-D005

IMPROVING HUMAN STEM CELL CRYOPRESERVATION USING SIMPLEX-LATTICE MIXTURE DESIGN

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KEY WORDS:
STEM CELL / CRYOPRESERVATION / SIMPLEX-LATTICE

BACKGROUND AND NOVELTY:
Cryopreservation is critical for the storage and transportation of human cell therapies. Effective cryopreservation must protect cells against freezing stresses with minimal toxicity. Freezing medium components significantly affect cryopreservation process efficacy. Cryoprotectants (CPAs, e.g. dimethylsulfoxide (DMSO) and glycerol) vary in effectiveness and their time, concentration and temperature dependent cytotoxicity. CPAs can be more effective and less toxic when combined. However, an understanding of how different CPAs interact to influence overall cryopreservation efficacy, with respect to diverse cell therapies, is lacking. Here we use a series of Simplex-Lattice mixture designs to identify the synergistic relationships between various CPAs and DMSO when used in a model cryopreservation process with relevance to stem cell preservation.

EXPERIMENTAL APPROACH:
An augmented Simplex-lattice mixture design was used to screen candidate CPAs and diluents used to formulate cryopreservation media. Human osteosarcoma cells (HOS TE85), bone-marrow derived human mesenchymal stem cells (hMSCs) and human embryonic stem cells (hESCs) were cryopreserved with experimental cryopreservation media using a 1°C/min slow-freeze process. After cryostorage in liquid nitrogen vapour, cells were thawed, washed and seeded into 6-well plates. Cryopreservation efficacy was evaluated using 1 hour post-thaw attachment and metabolic activity (calcein-acetomethoxy assay) as surrogate indicators of cell fidelity.

RESULTS AND DISCUSSION:
We show the utility of the Simplex-Lattice approach for the development of improved freezing media. Our data allows the ranking of individual cryoprotectants in terms of efficacy and demonstrates their ability to replace DMSO as part of a blend. Similarly, we show how complex diluents, such as serum, can be replaced with chemically-defined diluents without compromising cryopreservation efficacy.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-D006

TRANSFER OF MESENCHYMAL STEM CELL CULTURE PROCESS FROM MULTIPLE-TRAY STACKS TO THE INTEGRITY™ XPANSION™ MULTIPLATE BIOREACTOR

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KEY WORDS:
STEM CELLS / XPANSION / SINGLE-USE BIOREACTOR / SCALE-UP

BACKGROUND AND NOVELTY:
Cell therapy is offering a promising future in medical advances. While multilayer trays for cell amplification are suitable for R&D and preclinical purposes, they cannot support the large-scale industrial production. This requires an efficient and robust process based on “good manufacturing practice” (GMP).
Xpansion™ Multiplate Bioreactors have been designed to enable easy transfer from existing multiple-tray-stack processes by offering the same cell growth environment on 2D hydrophylized Polystyrene (PS) plates in a compact and closed system. Preserving the cell culture environment from one platform to the next is critical as small variations in physicochemical parameters, such as surface characteristics, pH and DO, can heavily impact stem cell growth and behavior.

EXPERIMENTAL APPROACH:
The Xpansion-10 plates (0.6 m2) is used first as a scale-down model to screen critical parameters and best conditions (cell attachment, growth and cell harvesting, and bioreactor homogeneity) before scaling up the process. Cell growth is followed by metabolites analysis and by microscopic observations (iLine microscope, Ovizio). Success criteria are finally defined by cell quality analyzed through QC tests.
Next, scale-up was assessed on Xpansion-50 plates (3.05m2) before achieving the final production scale using Xpansion-200 plates (12.2m2).

RESULTS AND DISCUSSION:
Mesenchymal stem cells were used as model. The process transfer was applied from Cell Stack (CS, Corning) to the Xpansion System by mimicking CS culture conditions. Data showed a similar QC results and cell growth at all scales of the Xpansion systems compared to the CS (max. confluence of ~30,000 cells/cm2, viability > 90%). Final scale Xpansion-200 plates (12.2 m2) will lead to a production of ~3.0x109 cells. Process transfer from CS to the Xpansion platform was successful. Cells retained their therapeutic potency. Reproducibility and reliability, as well as the feasibility of using Xpansion bioreactors as a tool for cell amplification, were demonstrated.

REF-D007

NEW PEPTIDE-BASED AND ANIMAL-FREE COATINGS FOR ANIMAL CELL CULTURE IN BIOREACTORS

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KEY WORDS:
SELF-ASSEMBLING HYDROGELS / RGD RECOGNITION / CELL ADHESION / COATING / BIOREACTOR SURFACE

BACKGROUND AND NOVELTY:
This work aims at designing animal-free, chemically defined and industrially scalable coatings for animal cell culture in bioreactors, as an alternative to collagen, fibronectin or Matrigel® for laboratory applications and industrial large scale cell productions. They are based on self-assembling short peptides bearing short polyethylene glycol moieties, functionalized with adhesion bioactive sequences to coat polystyrene or polyethylene terephthalate surfaces with a hydrogel resulting from the self-assembling of peptides into a network. RDG or other sequences embedded into the nanofibers network should be recognized by cells, which should favor their anchorage and spreading.

EXPERIMENTAL APPROACH:
- Liquid phase synthesis, purification and analytical characterization of PEGylated short self-assembling octapeptides.
- Synthesis of a RGD derived sequence called hRGD, by replacing Arg (R) by homoarginine (hR), which reduces proteolytic degradation while maintaining cell recognition.
- In cell adhesion tests, MRC5, Caco-2, CHO and human stem cells were incubated for 4h, washed, then fixed (2% formaldéhyde) and stained with rhodamine-phalloidin and DAPI. Observation was made with a fluorescence microscope and image analysis used NIS software. Hydrogel-based coatings were compared with collagen or fibronectin ones.

RESULTS AND DISCUSSION:
The absence of cytotoxicity of the hydrogels was assessed on the various cell lines using the MTT assay as well as the absence of significant LDH release upon incubation. Results from cytocompatibility studies and dose response effects with several cell lines including human stem cells allowed to evaluate the effect of this chemically defined animal-free peptide based coating vs. animal containing coating such as fibronectin. A first evaluation indicated that this compound favors cells adhesion and growth. This chemically defined animal-free peptide based coating is promising for next development of bioreactors with specific coating for animal as well as stem cells culture.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-D008

APPROACHES FOR AUTOMATIZED MSC EXPANSION AND DIFFERENTIATION OF HUMAN MSC IN SPECIALIZED BIOREACTORS

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KEY WORDS:
BIOREACTOR / MSC / EXPANSION / DIFFERENTIATION

BACKGROUND AND NOVELTY:
A main challenge in cell therapies and other tissue regeneration approaches is to produce a therapeutically significant cell number. For expansion of mesenchymal stem cells (MSC) the cultivation on 2D plastic surfaces is still the conventional procedure, even though the culture conditions differ significantly from the 3D environment in vivo. Additionally, static amplification of MSC is a labour-intensive procedure. We therefore used specialized bioreactors in order to maximize ex vivo expansion of MSC. Bioreactors furthermore enable integration of sensors for online monitoring of various parameters (e.g. pH, pO2, pCO2) and hence, allow ensured cultivation under well controlled and reproducible conditions. Beside cell expansion, directed differentiation of MSC was also achieved in bioreactors. Cells lack the ability to grow in 3D direction and build functional tissue in vitro. Thus, it is necessary to seed and culture cells on 3D matrices to obtain functional implants. For guided differentiation towards the osteogenic lineage, miniaturized perfusion bioreactors were developed.

EXPERIMENTAL APPROACH:
MSC, derived from umbilical cord and human adipose tissue, were used for the study. MSC were expanded under dynamic conditions in specialized bioreactors, and subsequently examined regarding their proliferations capacity, senescence and differentiation potential. After expansion cells were seeded onto 3D matrices. Osteogenic differentiation was induced under dynamic conditions. Status of differentiation was examined using different histological stainings.

RESULTS AND DISCUSSION:
Cell expansion in specialized bioreactor devices provides a high number of MSC, maintaining their stem cell properties. Cultivation in perfusion bioreactors promotes osteogenic differentiation. Thus our results support the argument that the application of tailor-made bioreactors are an essential step toward the production of stem cell based therapeutics and tissue engineering products.

REF-D009

STEM CELL ENGINEERING APPROACHES TOWARDS THE OPTIMIZATION OF THE EX-VIVO EXPANSION OF HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS FOR CELLULAR THERAPIES

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KEY WORDS:
UMBILICAL CORD BLOOD / HEMATOPOIETIC STEM/PROGENITOR CELLS / EX-VIVO EXPANSION / BIOENGINEERING / HEMATOPOIETIC CELL TRANSPLANTATION

BACKGROUND AND NOVELTY:
Umbilical Cord Blood (UCB) transplantation has faced a significant increase recently due to the unique features of UCB hematopoietic stem/progenitor cells (HSC) for the treatment of blood-related disorders. However, the low cell numbers available per unit significantly impair its widespread use for transplantation of adult patients. We have been focused on using rational stem cell engineering approaches targeting the maximization of UCB HSC expansion.

EXPERIMENTAL APPROACH:
Human UCB CD34+-enriched cells were co-cultured with human bone marrow (BM) mesenchymal stem cell (MSC)-derived feeder layers using a cytokine-supplemented serum-free medium. Several parameters were studied namely different initial stem/progenitor content, cytokine/growth factor cocktails and the impact of low oxygen tension values (2-10%) compared to normoxia (21%O2). Importantly, a dynamic co-culture system using plastic microcarrier-immobilized human BM MSC was evaluated to support expansion of UCB CD34+-enriched cells.

RESULTS AND DISCUSSION:
A design of experiments strategy was applied to the optimization of serum-free media formulations and cytokine cocktails, resulting in an increased cell productivity with a reduction in culture costs by 50-65% compared to previously established protocols. The impact of the initial CD34+ cell content was studied showing that a high (>90% CD34+ cells) initial progenitor content was not mandatory to successfully expand HSC. The effect of physiological O2 levels (5-10%) were found to be beneficial for an efficient expansion of UCB HSC. Concerning the expansion performed under dynamic conditions in spinner flasks, clinical meaningful CD34+ cell doses – 19 millions – were produced for a putative transplantation strategy of an adult patient, in a short time period (10 days). Overall, our results provide the basis for the establishment of efficient and controlled scalable culture systems for the generation of clinically significant cell numbers for cellular therapies.

REF-D010

INFLUENCE OF CA2+ AND MG2+ CONTENT IN CELL CULTURE MEDIUM ON CHO CELLS BEHAVIOR

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KEY WORDS:
CELL AGGREGATION / CELL ADHESION / CA2+, MG2+ CONCENTRATION / CHO CELLS

BACKGROUND AND NOVELTY:
Surface adhesion and aggregation are undesirable properties of CHO cells during industrial processes. There have been some prior reports suggesting the role of divalent ions on cell adhering behavior, pointing mainly to the Ca2+ content in the cell culture medium. In this work we explore the effect of different levels of Ca2+ and Mg2+ on CHO cells adhering properties and clumps formation under serum-free cultivation. The influence of previous cultivation conditions (stationary or stirred) on the quantitative effect of the divalent ions is also investigated.

EXPERIMENTAL APPROACH:
CHO cells were previously adapted to suspension and cultivated in protein free medium. Cells were separated into two groups for stationary and stirred cell propagation, and then cells properties under different divalent ions concentrations were studied in 24-well plates for 8 days. Concentrations of Ca2+ and Mg2+ were experimentally evaluated from 0 to 0.2 mM and 0 to 0.5 mM, respectively, using a chemically-defined protein-free medium. The combination of simultaneous changes in the concentrations of both ions, were also investigated.

RESULTS AND DISCUSSION:
Quantitative response surfaces were obtained for adhesion as well as aggregation properties as a function of Ca2+ and Mg2+ concentration in the medium. Cell aggregation was observed to be strongly promoted with increased Ca2+ concentrations, resulting in big cells clusters with decreased viability, probably associated to limited mass transfer of nutrient and oxygen. Independent variations of Mg2+ concentration showed not significant effect on cell aggregation. Regarding cell adhesion properties, Mg2+ ions proved to have the dominant effect on stimulating surface growth and induction of fibroblast shape. Simultaneous increased concentrations of Ca2+ and Mg2+ confirmed the induction of clumping and adhering cell culture behavior. The effects observed as wells as the kinetics of these phenomena can be used for culture medium optimization, but also to facilitate cell separation processes.

REF-D011

CULTURE SUPPLEMENT EXTRACTED FROM RICE BRAN FOR BETTER SERUM-FREE CULTURE

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KEY WORDS:
RICE BRAN / SERUM-FREE / CHO / MSC / ISLET

BACKGROUND AND NOVELTY:
In mammalian cell culture, fetal bovine serum (FBS) and proteins including albumin (BSA) have been extensively added to culture media as growth factor. But mammal-derived factors are potent source of various infections such as abnormal prion and viruses, and so alternative supplement is eagerly required. The alternative must be chemically defined or obtained from plant, as well as should be produced in commercial quantities and stably supplied. As an alternative supplement, we focused on rice bran extract (RBE), by-product of milling in the production of refined white rice, because rice bran contains abundant nutrients and proteins as well as antioxidants and because rice is cultivated plant, indicative of huge and stable supply.

EXPERIMENTAL APPROACH:
RBE was extracted in alkaline solution and then precipitated with acid. The precipitate was freeze-dried.

RESULTS AND DISCUSSION:
Mitogenic activity of RBE was evaluated using cell lines. On growth and MoAb production of hybridoma in serum-free medium, desired effects of RBE were observed and the effect was superior to BSA. Similarly, serum-free culture of CHO-DP12 added with RBE exhibited increased cell growth and production. Growth of HepG2 and HeLa cells in the serum-free medium was also improved. Together all, RBE had mitogenic activity on various cell lines. As primary cells, MSCs from Wistar rat were expanded in serum-free medium with RBE or without and then the medium was changed into osteoblast-inducing medium. While MSC expanded in the serum-free medium lost it, the cells expanded in the presence of RB retained the potency, suggesting that RBE contains physiologically active substances maintaining potency of differentiation during ex vivo serum-free culture. Pancreatic islets, isolated from Lewis rats, were also tested in the presence of RB. While islets died out by one week in basal medium, islets successfully survived in the presence of RB. This result supports that RBE could alternate FBS in islets culture.

REF-D012

CELL PROLIFERATION OF HUMAN MESENCHYMAL STROMAL CELL IN A DISPOSABLE BIOREACTOR

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KEY WORDS:
HUMAN MESENCHYMAL STROMAL CELL / BIOREACTOR / CELLULAR THERAPY

BACKGROUND AND NOVELTY:
Mesenchymal stromal cells (MSC) have been exploited in numerous clinical trials to investigate their potential in immune regulation, hematopoiesis and tissue regeneration [1]. To meet the increasing clinical demand, the use of bioreactors is a viable option to ensure efficient and reliable ex vivo expansion. The objective of this work was to develop a method to verify the feasibility of using a disposable bioreactor (Fibrastage®) for the successful expansion and recovery of human MSC, preserving their biological properties for cellular therapy applications.

EXPERIMENTAL APPROACH:
MSC derived from umbilical cord blood (n=3, three different donors) were seeded in 75 cm2 T-flasks in a-MEM culture medium supplemented with 10% v/v of Fetal Bovine Serum (FBS), until obtaining 1.2x105 cells/mL. Cells were inoculated in a pre-sterilized disposable cell culture bottle loaded with 10 grams of Fibra-cel disks and 500 mL culture medium. The cell concentration and cell viability were detected, also daily, by MTT assay in the Fibra-cel disks. After 7 days of culture, the cells were harvested and the immunophenotypic properties and differentiation potential (osteogenic and adipogenic) were determined.

RESULTS AND DISCUSSION:
The maximum cell density of 4.15(±0.810)x108cells was obtained at day 7; corresponding to 7,0(±1.4)-fold increase approximately. During the period in culture, glucose and glutamine concentrations did not decrease below 0.26 and 0.08 mM, respectively. As a result of glucose and glutamine consumption, lactate and ammonia were produced, reaching a maximum concentration of 10.4 and 1.3 mM at day 5, respectively, not considering inhibitory values. Expanded MSC retained their differentiation potential into adipogenic and osteogenic lineages. The results of immunophenotype analysis revealed that these expanded cells were positive for several markers common to MSC and, as expected, no significant difference in the percentage of positive cells was observed before and after cell expansion ($p>0.05$).

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REF-D013

NOVEL SCALABLE PLATFORMS FOR THE PRODUCTION OF PURE CARDIOMYOCYTES DERIVED FROM Ipsc

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BACKGROUND AND NOVELTY:

The production of cardiomyocytes (CM) from induced pluripotent stem cells (iPSC) holds great promise for autologous regeneration therapies, patient-specific disease modeling and cardiotoxicity testing [1]. However, the widespread clinical and industrial use of these cells is still hampered by the lack of robust bioprocesses for the production of CM in high purity, consistent quality and relevant quantities [2]. The goal of this study was to develop a scalable platform for the production of pure iPSC-derived CM. Our strategy consisted in designing an integrated bioprocess for CM differentiation and purification in environmentally controlled bioreactors, where the necessary conditions to control stem cell fate are thoroughly tuned [3].

EXPERIMENTAL APPROACH:

A transgenic aPIG murine iPSC line, in which the cardiac-restricted a-myosin heavy chain promoter drives both eGFP and puromycin resistance gene expression, was used. iPSC were cultivated as aggregates in distinct bioreactor systems (stirred tank and Wave™ bioreactors) and the impact of cyclic mechanical strains, promoted by different stirring profiles, on CM differentiation was evaluated. The differentiation process was monitored using flow cytometry, qRT-PCR and microscopy analysis.

RESULTS AND DISCUSSION:

Our results showed that stirring is a key parameter in iPSC differentiation towards functional CM. Using an intermittent stirring profile in stirred tank bioreactors, we were able to improve the differentiation yields (up to 43 CM per initial iPSC) and the final purity of CM (>97%). Furthermore, we show for the first time that wave-induced agitation of CellbagTM bioreactors enhances CM differentiation. CM-committed cells were identified earlier and a higher differentiation yield was achieved (60 CM per iPSC) when compared to stirred tank bioreactor cultures.
The bioprocesses developed herein provide important insights for the establishment of robust iPSC-derived CM production platforms, paving the way for the implementation of novel cell-based therapies.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

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REF-D014

TOWARDS A CLINICAL-GRADE BIOPROCESS FOR THE LARGE-SCALE PRODUCTION OF HUMAN PLURIPOTENT STEM CELLS

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BACKGROUND AND NOVELTY:

Human embryonic stem cells (hESCs), with their unique characteristics for indefinite proliferation and pluripotency, are an appealing source for cell replacement therapies, tissue engineering and in vitro toxicology applications. For the clinical implementation of these cells, there is the need for translating the culture protocols developed at research laboratories into validated manufacturing platforms that can guarantee reproducibility, scalability, standardization, robustness and safety [1]. Within this context, the main aim of this work was to establish a clinical-grade bioprocess for the large scale production of undifferentiated hESCs in fully defined conditions.

EXPERIMENTAL APPROACH:

Different culture medium formulations and several types of synthetic microcarriers (Corning) were evaluated for their biocompatibility and ability to support hESC (Cellartis AB, Cellectis Stem Cells,) expansion in stirred tank bioreactor systems. hESC cultures were monitored daily in terms of cell growth, viability and metabolic performance. The undifferentiated phenotype and pluripotency of hESCs during expansion was confirmed using immunofluorescence microscopy, flow cytometry and RT-PCR tools and in vitro pluripotency assays.

RESULTS AND DISCUSSION:

Synthetic microcarriers showed to be very effective to support hESC expansion in stirred tank bioreactors, allowing higher microcarrier colonization (up to 90%) and fold increase in cell concentration when compared to standard cultures using matrigel-coated microcarriers [2], while maintaining cell undifferentiated phenotype and pluripotency. Additionally, the combination of upstream and downstream strategies has been evaluated, aiming at establishing a robust and integrated bioprocess to generate hESC-based products in relevant quantities and in high purity and quality to satisfy the clinical demands.

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REF-D015

CONTROLLING HOST-CELL BASED PROTEOLYTIC ACTIVITY IN CHO CULTURES: ROLE OF DIFFERENT CULTURE PARAMETERS?

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KEY WORDS:
PROTEOLYTIC ACTIVITY / VACCINE DEVELOPMENT / CHO CELL CULTURE

BACKGROUND AND NOVELTY:
Mammalian cell cultures are integral to the production of recombinant proteins. A common problem encountered in cell culture processes is clipping of the target product by host cell proteases. We observed significant proteolytic activity (>60% target product clipped) in a CHO based production process used to produce a heavily glycosylated subunit vaccine candidate. By systematically characterizing this proteolytic activity under various culture conditions, we developed a cell culture based process where the proteolytic clipping could be significantly reduced resulting in less than 5% of the product being clipped. Here we present our strategy to characterize and control this proteolytic activity.

EXPERIMENTAL APPROACH:
Based on sequencing of the clipped fragment and inhibitor screenings we established that the enzyme responsible for observed proteolytic clipping belongs to a serine protease family. We performed a systematic investigation to understand the effect of different culture parameters, namely host cell line, culture media, culture pH, culture temperature, culture duration and chemical and protein/peptide based inhibitor supplementation on the proteolytic activity in the culture. We used commercially available assays to measure the proteolytic activity directly in the cell culture samples and established that the detected activity in culture directly correlated with the amount of clipped material in purified product.

RESULTS AND DISCUSSION:
Based on our observations, we established that different culture parameters affect the proteolytic clipping to varying degrees in our target product. We identified cell culture suitable protease inhibitors whose supplementation during culture phase effectively reduced proteolytic clipping without impacting the cell growth negatively. By optimizing the inhibitor feeding strategy during culture phase, we developed a CHO based production process that consistently produced the target protein with desired quality attributes and minimal proteolytic clipping.

REF-D016

EXTENDED GENE EXPRESSION BY REPEATED TRANSIENT TRANSFECTION OF HEK 293 SUSPENSION CELL CULTURES

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KEY WORDS:
TRANSIENT TRANSFECTION / VIRUS-LIKE PARTICLES / PRODUCTION

BACKGROUND AND NOVELTY:
Production of recombinant products in mammalian cell cultures is currently achieved by stable gene expression (SGE) or transient gene expression (TGE). The former is based on the integration of a plasmid DNA into the host cell genome allowing continuous gene expression. The latter is based on episomal plasmid DNA expression. Since episomal DNA is lost as cells divide, the production period typically lasts less than 96 hours; thus, limiting production capacity. A novel gene expression approach termed extended gene expression (EGE) was explored in this study. The aim of EGE is to prolong the production period by repeatidly transfecting cell cultures. The benefits of this method were evaluated for the production of Gag virus-like particles (VLPs) in HEK 293 cell cultures.

EXPERIMENTAL APPROACH:
Suspension HEK 293 cells were grown in Freestyle medium containing non-animal derived additives. Cultures were transfected with 25-kDa linear PEI. The initial transfection round was typically carried out when cells reached 2E06 cells/mL using 1 µg of plasmid DNA/mL of cell culture as performed using our standard TGE protocol. Transfections were repeated using different plasmid DNA concentrations, intervals of time and culture feeding conditions in order to identify the best approach to achieve sustained high level expression.

RESULTS AND DISCUSSION:
Using a standard TGE protocol, Gag VLPs are typically recovered from cell culture supernatants 4 days after transfection obtaining 51.3 µg of Gag, which is equivalent to 1.5E11 VLPs. To maintain VLP production during sequential cell divisions, a EGE strategy that involves repeated rounds of transfection was explored. Best production performance was achieved by re-transfecting cell cultures every 48 hours using a DNA concentration of 0,5 µg/mL and medium exchange. Using this strategy, production time was prolonged to 10 days. During this time, cell viability and the percentage of transduced cells was maintained high. Importantly VLP production was increased 6-fold.

REF-D017

CONTINUOUS PRODUCTION OF INFLUENZA VIRUSES USING AVIAN SUSPENSION CELLS

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KEY WORDS:
CONTINUOUS PRODUCTION / INFLUENZA VACCINES / DEFECTIVE INTERFERING PARTICLES / AGE1.CR CELLS

BACKGROUND AND NOVELTY:
Influenza viruses represent a high burden for human health during seasonal epidemics and are a continuous threat due to their potential to cause pandemics. The best protection against the contagious respiratory illness caused by influenza viruses is annual vaccination. However, the current production capacities for influenza vaccines are insufficient to meet the increasing global demand. Therefore, we explored the possibility to establish a continuous production process for influenza viruses using avian suspension cells.

EXPERIMENTAL APPROACH:
To propagate influenza viruses in continuous culture a two-stage bioreactor set-up was used. Thereby, AGE1.CR cells (duck-derived suspension cell line from the ProBioGen AG, Germany) were cultivated in the first stirred-tank bioreactor (STR). Here, an almost constant cell concentration of 3x106 cells per mL was maintained and cells were constantly fed to the second STR where the influenza virus infection took place.

RESULTS AND DISCUSSION:
Using this two-stage reactor system it was possible to continuously produce influenza viruses. However, the subsequent virus titer analysis revealed a periodic increase and decrease of virus titers during the run-time of 17 days. These titer fluctuations were caused by the accumulation of defective interfering particles (DIPs), which were detectable by PCR. With increasing amounts of DIPs the concentration of infectious viruses starts to decrease. In turn, total virus titers also decline since DIPs are unable to replicate without complete helper viruses. Consequently, the perfusion rate dilutes both infectious viruses as well as DIPs and at a low multiplicity of infection virus replication is able to restore. In the future, this system shall be analyzed in more detail for its suitability to produce influenza viruses and to study viral evolution as well as the generation of DIPs.

REF-D018

CHARACTERIZATION AND QUANTIFICATION OF FLUORESCENT GAG VIRUS-LIKE PARTICLES

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KEY WORDS:
VIRUS-LIKE PARTICLES / VACCINES / QUANTIFICATION / CHARACTERIZATION / FLUORESCENCE

BACKGROUND AND NOVELTY:
Upon expression, the Gag polyprotein of HIV-1 spontaneously assembles giving rise to enveloped virus-like particles (VLPs). These particulate immunogens offer great promise as HIV-1 vaccines. In order to develop robust VLP manufacturing processes, the availability of simple, fast and reliable quantification tools is crucial. Traditionally, commercial p24 ELISA kits are used to estimate Gag VLP concentrations. However, this quantification technique is time-consuming, laborious, costly and prone to methodological variability. Reporter proteins are frequently used during process development to allow a straightforward monitoring and quantification of labeled products. This alternative was evaluated in this work.

EXPERIMENTAL APPROACH:
Generation of fluorescent VLPs was carried out by transient transfection of HEK 293 suspension cells with a plasmid coding for Gag fused to GFP. VLP budding from producer cells was evaluated by microscopy. SDS-PAGE, Western blot, nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) were used to characterize the VLPs. A fluorescence-based VLP quantification method was developed and validated. To verify method reliability, the estimated titers were compared with those obtained by densitometry, ELISA, TEM and NTA.

RESULTS AND DISCUSSION:
Correct assembly and budding of Gag VLPs into the cell culture supernatant was observed upon transfection. Spherical virus particles surrounded by a lipid envelope with an augmented diameter consistent with immature HIV particles (150 nm) were observed by TEM. The Gag-GFP polyprotein was the major component found in purified samples. The fluorescence-based quantification method developed was satisfactorily validated according to ICH guidelines. Importantly, it showed to be useful for analysis of both crude and purified samples. Fluorescence-based titers were in good agreement with those obtained by p24 ELISA as well as TEM and NTA analyses. The method was successfully applied to study VLP production.

APPLICATION OF SINGLE-USE BIOREACTORS FOR CULTURING ADHERENT CELLS FOR VACCINE APPLICATIONS

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KEY WORDS:
SINGLE-USE BIOREACTOR / CELL CULTURE / VACCINE / MICROCARRIER

BACKGROUND AND NOVELTY:
Single-use bioreactor technology is an effective tool for performing process development studies and for producing clinical and commercial material at a range of volumes. Here we show the utility of 3L and 50L single-use stirred tank bioreactors for vaccine applications requiring the cultivation of adherent mammalian cells on microcarriers.

EXPERIMENTAL APPROACH:
A study was conducted with two types of microcarriers to assess feasibility of the mixing system under various power per unit volume conditions in 3L and 50L single-use stirred tank bioreactor.

RESULTS AND DISCUSSION:
The normalized mixing speeds required to achieve adequate suspension of the microcarriers at 3L and 50L were then determined. Further study demonstrated that efficient MDCK cell growth is feasible at 3L and 50L scale.

IMPROVING VACCINIA VIRUS PRODUCTIVITY IN HELA CELLS BY OPTIMIZATION OF BIOPROCESS STRATEGY

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KEY WORDS:
BIOPROCESS STRATEGY / VACCINIA VIRUS / HELA CELLS / PRODUCTIVITY

BACKGROUND AND NOVELTY:
Virus productivity development is in constant evolution, driven by demand for higher virus yields, shorter timelines and global regulatory requirements. In the past few years, HeLa cells have been employed for many purposes such as vaccine production. Within the prospect of a new vaccine manufacturing process, we have developed suspended HeLa cells in serum free culture media process.

EXPERIMENTAL APPROACH:
The development of an efficient process for production of vaccinia virus (VV) in HeLa cells was performed in 3 stages. Firstly, the adaptation of cells to suspension was realized according to 3 strategies with a panel of serum free media(SFM). Direct adaptation to suspension was carried out with or without progressive serum deprivation. Afterwards, sequential adaptation was realized from serum containing medium to SFM and to suspension, at the same time. After 20 passages we noticed HeLa cells are adapted to growth in suspension in the selected SFM. However, we observed a decrease of virus productivity with adapted cells to suspension compared to adherent cells in serum supplemented medium. Secondly, the virus productivity was imroved by a screening of dedicated media for infection. To monitore their impact, we used a VV expressing the Green Fluorescent Protein for flow cytometry analysis and this was done in parallel with titration. At the end of this study we observed an increase of ten fold of viral productivity linked directly to the medium used. To finish, thanks to the media screeening we were able to identifiy one component with significant negative impact on viral productivity: the Dextran Sulfate.

RESULTS AND DISCUSSION:
During the study, the selection of the medium was identified as particularly important and should be carefully considered in the process of virus production in serum free media. Indeed, some media may contain components, infection inhibitors, such as the Dextran Sulfate. These results constitute valuable information for the development of cell culture process for VV production.

REF-D021

DEVELOPMENT OF A PRODUCTION PROCESS FOR INFLUENZA VLPS IN HEK 293SF CELLS USING THE BACMAM SYSTEM

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KEY WORDS:
VIRUS LIKE PARTICLES / INFLUENZA / VACCINE / RECOMBINANT PROTEIN / BACULOVIRUS

BACKGROUND AND NOVELTY:
Influenza virus-like particle (VLP) vaccines are one of the most promising approaches to respond to the threat of future influenza pandemics. VLPs are similar to live attenuated vaccines and exhibit an enhanced safety profile, as they are composed only of viral antigens rendering them non-infectious. Past Influenza VLP studies mainly performed in the baculovirus-insect cell system report contamination with recombinant baculovirus that are difficult to remove during purification. Baculovirus with mammalian promoters (Bacmam) have been shown to efficiently transduce mammalian cells and further express genes but are unable to replicate. In order to address the issue of baculovirus contamination, VLP production was performed in HEK 293SF cells using the Bacmam gene delivery system. The proposed system was assessed for its ability to produce influenza VLPs composed of HA, NA and M1.

EXPERIMENTAL APPROACH:
Process parameters, such as MOI, additives, time of harvest and preliminary recovery steps were explored in this study. VLP purification was completed with either differential centrifugation steps or ultrafiltration using a 100-kDA cut off. Influenza proteins were detected by western blot and hemagglutination assay.

RESULTS AND DISCUSSION:
It is widely assumed that during influenza VLP production functional particles bud from the cell membrane, much like their parent virus. Production of influenza VLPs in HEK 293 cells has given indication that there may not be such a direct correlation to influenza with respect to VLP budding and functionality. Detection of VLPs in the supernatant is found in low amounts compared to what is left associated with or inside the cell pellet at harvest. The question of whether these non-released particles are intact, how to best release them from the cells to increase final product yield and their functionality was explored during this study.

REF-D022

EFFICIENT PRODUCTION OF VIRAL ENVELOPE PROTEINS IN RECOMBINANT INSECT CELLS

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KEY WORDS:
INSECT CELL / RECOMBINANT PROTEIN PRODUCTION / VIRUS-LIKE PARTICLE

BACKGROUND AND NOVELTY:
The baculovirus–insect cell system has been used extensively for the production of recombinant subunit vaccines including virus-like particles (VLPs), but continuous production is virtually impossible due to the lytic nature of the baculovirus infection process. Stably transformed insect cell lines can be employed as an attractive alternative for the continuous production of complex recombinant proteins. In the present study, the production of Japanese encephalitis virus (JEV) envelope proteins in stably transformed lepidopteran insect cells was investigated.

EXPERIMENTAL APPROACH:
The DNA fragment encoding the JEV prM signal peptide, the precursor (prM) of the viral membrane protein (M), and the envelope glycoprotein (E) was cloned into the plasmid vector pIHAbla. The pIHAbla contained the silkmoth actin promoter downstream of the baculovirus IE-1 transactivator and the baculovirus HR3 enhancer for high-level expression, together with a blasticidin resistance gene for use as a selectable marker. After transfection with the resultant plasmid, Trichoplusia ni BTI-TN-5B1-4 (High Five) cells were cultivated with blasticidin, and antibiotic-resistant cells were obtained.

RESULTS AND DISCUSSION:
Western blot analysis and enzyme-linked immunosorbent assay (ELISA) of a culture supernatant showed that recombinant High Five cells secreted an E antigen equivalent to the authentic JEV E. Sucrose density-gradient sedimentation analysis of the culture supernatant suggested that secreted E antigen molecules were in a particulate form. VLPs recovered from the supernatant successfully induced neutralizing antibodies in mice, particularly when adsorbed to alum adjuvant. High yields (> 10 mg/L) of E antigen were achieved in serum-free suspension cultures. These results indicate that recombinant insect cells may offer a novel approach for efficient production of VLPs for use as vaccines and diagnostic antigens.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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CELL CULTURE-BASED INFLUENZA VIRUS PRODUCTION IN HOLLOW FIBER BIOREACTORS

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KEY WORDS:
INFLUENZA VACCINE / HOLLOW FIBER BIOREACTOR / CELL SPECIFIC PRODUCTIVITY

BACKGROUND AND NOVELTY:

Cell retention and high density perfusion culture are the next challenges to significantly increase product yields in influenza vaccine manufacturing. In this scenario, the incorporation of hollow fiber bioreactors (HFBRs) has been only recently proposed. HFBRs have been extensively and successfully studied in the past for recombinant protein production. The outstanding productivities achieved in these bioreactors, suggest the use of HFBRs for vaccine production to be an attractive option. In this work, the feasibility of influenza A virus production in a HFBR was explored for MDCK cells.

EXPERIMENTAL APPROACH:

The small scale single-use hollow fiber bioreactor PRIMER HF® (Biovest International) was characterized for cultivation of adherent and suspension MDCK cells. Both cell lines were cultured in the extracapillary space (ECS) (50 mL) of the hollow fibers and infected with the A/PR/4/38 (H1N1) and the pandemic A/Mexico/4108/2009 (H1N1) influenza virus strain. Virions were collected by multiple consecutive complete harvests (50 mL) of the ECS, and quantified by hemagglutination (HA) and TCID50 (infectious virus) assay. Different infection and harvest strategies were evaluated.

RESULTS AND DISCUSSION:

By following a multiple harvest strategy, very high HA titers were observed with a maximum of 3.4 and 3.8 log10 HA units/ 100 µL for adherent and suspension MDCK cells, respectively. As cell concentrations are difficult to determine in these bioreactors, a major challenge lies in the optimization of the "time of infection". The cell-specific virus productivity, estimated from the average number of released cells in harvests and after the final trypsinization, was approx. 14 000 virions/cell for both cell lines. This is similar to values reported for other bioreactors. Work is in progress to optimize productivity and evaluate whether all cells present in the HFBR can be infected faster but equally efficient in a larger, more automated HFBR system (AutovaxID), that allow more fluid movement within the ECS space.

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IMPROVED HEK 293-FLEX CELLS FOR A BETTER HCV VACCINE CANDIDATE

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KEY WORDS:
VACCINE / RETROVIRUS LIKE PARTICLES / HEPATITIS C / IMMUNOGENICITY / HEK293-FLEX

BACKGROUND AND NOVELTY:

Hepatitis C virus (HCV), together with HIV and Malaria, is one of the three severe human pathogens for which a vaccine is still lacking. Over the past decade intensive efforts were made towards the development of a suitable HCV vaccine and correspondent production system; several options are described in the literature. Horizontal to these systems are two main issues to be addressed. First, the immunogenicity of the particles related with host proteins incorporation after budding (1) and secondly, the production yields (2). Here we present a modified high-titer retroviral vector producer cell line for the manufacture of HCV pseudotyped retroVLPs with low non-specific immunogenicity.

EXPERIMENTAL APPROACH:

HEK293-FLEX (3-4) cells were tagged with a second site-specific cassette replacement system using the Cre/Lox enzyme/site. This construct encodes for recombinant eGFP protein allowing a rapid screen for high expression clones by flow cytometry. After isolation of several clones, analysis of eGFP expression, we checked for single copy integration by real-time qPCR. One clone was then selected to express both HCV structural proteins E1/E2 and a HCV E1 engineered protein with increased cell surface localization. To reduce immunogenicity issues, retroVLPs producer cells were silenced for CD81, a host protein incorporated onto retroVLPs that contributes for an undesired immune response.

RESULTS AND DISCUSSION:

Purified retroVLPs were analyzed by ELISA, western-blot and mass spectrometry for CD81, E1 and E2 incorporation and quantification. A knock-down of 99% of CD81 incorporation together with a higher incorporation of HCV E1 and E2 on retroVLPs was observed. In parallel, CD81-specific antibody and T-cell responses were analyzed in mice immunized with CD81 positive and negative retroVLPs; no major difference was observed between study groups. This novel cell line for the production of retrovirus based biopharmaceuticals will be further improved to develop a new prophylactic and therapeutic HCV vaccine candidate.

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VERO CELLS CULTURE AND SABIN POLIO VIRUS REPLICATION IN AN ANIMAL COMPONENT FREE MEDIUM

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KEY WORDS:
VERO CELLS / IPT-AFM MEDIUM / SABIN POLIO VIRUS

BACKGROUND AND NOVELTY:

Vero cells have been widely used in the manufacturing of viral vaccines such us rabies, polio and influenza vaccines. IPT-AFM is an animal component free medium developed at the viral vaccines R&D unit of Institut Pasteur de Tunis, Tunisia (Rourou et al., 2009a) for Vero cells culture and rabies vaccine production in stirred cultures using Cytodex 1 microcarriers (Rourou et al., 2009b). This medium has a simple composition and a low cost compared to commercially available serum free media.

EXPERIMENTAL APPROACH:

Recently, Intravacc (former part of RIVM) initiated the development of Sabin-IPV which is an injectable and formalin-Inactivated Polio Vaccine, based on the use of attenuated 'Sabin' polio virus strains. The project was started essentially to respond to WHO's call for new polio vaccines in the framework of polio eradication strategy (Bakker et al., 2011).

RESULTS AND DISCUSSION:

In the current work, the potential use of IPT-AFM for future Sabin-IPV manufacturing was evaluated. Preliminary experiments have taken place by first studying Vero cell growth in a new formulation of IPT-AFM, containing a reduced number of plant hydrolysates. In addition, kinetics of cell growth and Sabin Polio virus type 1 replication in Vero cells cultivated using IPT-AFM, a commercially available animal component free medium and serum containing medium were assessed in spinner flasks. Finally, polio virus production in stirred bioreactor operating in batch mode was studied using IPT-AFM.

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AN EXAMINATION OF CELL CULTURE PERFORMANCE ON MULTI-LAYER ADHERENT CELL CULTURE SYSTEMS

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KEY WORDS:
CELL FACTORY / MULTI-LAYER / ADHERENT / CELL CULTURE

BACKGROUND AND NOVELTY:

For decades, multi-layered cell culture systems have been a reliable tool for the production of vaccines, recombinant proteins, and for the generation of cell mass. Multi-layered systems maximize the volume for a given footprint. However, due to the increase in surface area and multiple layer design, delayed cell attachment in the central area of the multi-layer system may result. In addition, the geometry of these systems have visual limitations for monitoring the cell performance on the middle layers. The purpose of this application study is to examine the uniformity of conditions and consistency of performance from layer to layer.

EXPERIMENTAL APPROACH:

Four cell lines were cultured in 10 layer Nunc Cell Factory Systems (CF10). The cell lines examined were CHO, VERO, MDBK, and MDCK. The pooled cells were prepared in 10% FBS pre-warmed to 37°C. The CF10's were incubated with vent closed in a warm room at 37°C without CO2 control for 4 days. The medium in each CF10 was then discarded and the cells were stained with crystal violet. The layers were separated with a hot knife, and each individual layer was photographed. In addition, photomicrographs were taken at three points on each layer.

RESULTS AND DISCUSSION:

Photographs and photomicrographs of the individual layers from two CF10's were compared side by side for each cell line. Cell growth was similar on all layers except minor patterns on the bottom layers attributable to a shelf-pattern which is common in cell culture. These observations suggest that for most cell lines there is minimal variation in cell culture performance from layer to layer in a 10-layer device.

REF-D027

CHARACTERIZATION OF OXYGEN TRANSFER, POWER INPUT AND HYDRODYNAMICS IN ORBITALLY SHAKEN SINGLE-USE BIOREACTORS FOR ANIMAL AND PLANT CELL CULTIVATION

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KEY WORDS:
MAXIMUM OXYGEN TRANSFER CAPACITY / PRODUCTION OF RECOMBINANT PROTEINS / POWER INPUT / SCALE-UP / SHAKEN SINGLE-USE BIOREACTOR

BACKGROUND AND NOVELTY:
Orbitally shaken single-use bioreactors are surface aerated and, therefore, combine the advantages of two established systems: the flexibility and process safety of single-use equipment with the reduced contamination risk and cost-efficiency of surface aerated bioreactors. Unlike conventional well-established stirred tank reactors, however, such single-use bioreactors have not yet been precisely characterized. Detailed knowledge about critical engineering parameters such as the maximum oxygen transfer capacity, the power input and fluid dynamics are essential requirements for the reliable application of these novel reactor systems.

EXPERIMENTAL APPROACH:
A comprehensive characterization of shaken single-use bioreactors with nominal volumes from 50 mL to 200 L was conducted in this study. Innovative measuring techniques for the determination of the maximum oxygen transfer capacity, power input and fluid dynamics were used to optimize cultivation conditions for animal and plant cell suspensions. A liquid distribution model was implemented in Matlab to calculate the hydrodynamics of the systems and Computational Fluid Dynamics (CFD) was used to validate the mathematical model. Finally, the mixing intensity was quantified by developing a scale and volume independent power input correlation.

RESULTS AND DISCUSSION:
Optimized conditions for the cultivation of animal and plant suspension cells were calculated using the developed power input correlation and the established liquid distribution model. The results indicated that orbitally shaken single-use reactor systems are ideally suited for the cultivation of slow growing animal-, plant- or and insect cells. Optimal process parameters can now be calculated easily with the established calculation tools. However, the results showed that these novel reactor systems are not suitable for the cultivation of fast growing bacteria or yeast cells with a high oxygen demand.

REF-D028

REAL-TIME MONITORING OF CRITICAL PHASES IN VIRAL PRODUCTION PROCESSES BY DIELECTRIC SPECTROSCOPY FOR ENVELOPED AND NON-ENVELOPED VIRUSES

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KEY WORDS:
PERMITTIVITY / VIRAL REPLICATION KINETICS / ON-LINE PROCESS MONITORING / MEMBRANE CAPACITANCE

BACKGROUND AND NOVELTY:
Cell culture-based viral vectors and vaccines manufacturing is an area of critical interest to many industries and public health agencies. Safety and cost-effectiveness, largely determined by processes, are critical success factors for any viral derived product for which efficacy has been demonstrated. Quality control of such products and processes being of high importance, the regulatory agencies strongly recommend to manufacturers implementation of on-line monitoring tools and of control strategies in their production lines. On-line dielectric spectroscopy is a technology accepted as a good indicator of culture state in cell-based systems. in the present work, we demonstrate that this tool can also monitor a wide range of viral productions kinetics in different operating conditions.

EXPERIMENTAL APPROACH:
The technology was assessed for different cell production platforms and with various production modes of enveloped and non-enveloped viruses. Evolution of capacitance signals (Demax, fc) and of cell dielectric properties (r, CM, si) were correlated with key viral replication phases (intracellular accumulation of viral components, viral assembly, viral release and cell death).

RESULTS AND DISCUSSION:
It was possible to identify typical patterns in capacitance signals and dielectric properties linked to viral biological events. Intracellular accumulation of viral capsid in the cell cytoplasm was associated with changes in CM and si properties, for all types of virus possessing a nucleocapsid. Moreover, a typical pattern of Demax, fc, CM and si was indicating onset of viral release in all the enveloped virus production studied. Differences in cell dielectric properties appear to be specific to the type of virus produced (enveloped/non-enveloped, viral size, assembly site), but, clearly the technology is generic and sufficiently robust to support on-line quality control in a wide variety of viral production cell-based processes.

REF-D030

DEVELOPMENT OF A FLOW CYTOMETRY-BASED ASSAY FOR THE QUANTIFICATION OF INFECTIOUS INFLUENZA VIRIONS

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KEY WORDS:
INFLUENZA VIRUS / INFECTIOUS VIRUS TITRATION / FLOW CYTOMETRY

BACKGROUND AND NOVELTY:
Commonly used methods for infectious virus titration (TCID50, plaque assay) are labor and time consuming and involve subjective interpretation of test results. Alternatively, faster methods using flow cytometry have been applied for different viruses [1-6]. Nevertheless, detection techniques are specific for each virus and corresponding assays have to be carefully validated. In particular, detection and quantification limits as well as sensitivity and robustness need to be characterized. Here, a flow cytometry-based method is presented, in which optimal conditions are determined for titration of different influenza virus strains.

EXPERIMENTAL APPROACH:
MDCK cells grown in multi-well plates were infected with serial dilutions of different influenza seed viruses. At selected incubation times (6-24 h) infected cells were stained with a FITC-labeled anti-influenza NP antibody and counted by flow cytometry. Correlation between flow cytometry-derived measurements and TCID50 titers was evaluated for a wide concentration range of three different virus strains. Finally, the assay was used for monitoring the production of a fourth strain in a bioreactor.

RESULTS AND DISCUSSION:
Cytometry-derived and TCID50 titers were comparable when an incubation time of 18-24 h post infection was selected, which offers some operational flexibility. A good correlation with TCID50 titers ($r^2 \geq 0.998$) was found for all tested virus strains. Additionally, a limit of quantification (LoQ) around 10^3 Infectious Units per mL (IU/mL) was observed for cytometry based assay, whereas a LoQ of 3.2×10^2 IU/mL has been determined for manual titrations [7]. Flow cytometry-derived titers of the virus strain produced in a bioreactor showed no significant difference with measurements obtained by TCID50 assay. Overall, we developed a fast flow cytometry based assay, which adds objectivity and reproducibility to the titration of influenza viruses.

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REF-D031

COMPARATIVE STUDY OF BLUETONGUE VIRUS SEROTYPE 8 PRODUCTION ON BHK-21 CELLS IN A 50L BIOSTAT® STR SINGLE-USE BIOREACTORS VS ROLLER BOTTLES

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KEY WORDS:
BLUETONGUE VIRUS / SEROTYPE 8 / BHK-21 CELL LINE / SINGLE USE BIOREACTOR

BACKGROUND AND NOVELTY:
Bluetongue (BT) is a major disease of ruminant livestock that can have a substantial impact on income and animal welfare.Pfizer Animal Health have licensed bluetongue vaccines able to prevent viremia. As an example, the use of the BTV-8 vaccine is routeinely produced in roller bottles (RB). The aim of this study is to investigate Single-use Bioreactor technology as an alternative to RB. This technology combines the basic concept of allowing the cells to attach to a surface (microcarriers) with the avantages of suspension, wich allows a better control of culture conditions and systematic and automatic culture process.

EXPERIMENTAL APPROACH:
Several studies were conducted to compare the growth of BHK-21 cells in RB and in a 50L single-use BIOSTAT® STR bioreactor (Sartorius-Stedim Biotech S.A). BHK-21 cells were grown in microcarriers in the STR bioreactor and the cell production was optimized with respect to pH, t^a, stirring speed and aeration rate. Once it was determined that the RB and microcarriers achieve maximum yield the next step was to evaluate the BTV-8 production. Cell growth and virus production in the STR bioreactor was conducted at the optimal conditions determined previously on conventional bioreactors.

RESULTS AND DISCUSSION:
Results prove that when using the 50L STR bioreactor, BHK-21 cells attached and grew efficiently on microcarriers. At confluency the cell concentration was equal or higher than in roller bottles. The antigen BTV-8 production using the said technology has been investigated. Similar or better results were obtained in infected microcarrier cultures when compare to RB. Improvements are explained by the increase in cell number per mL at the moment of infection, controlled regulation of pH and better oxygen supply. As a conclusion, BTV-8 antigen with satisfactory yields can be obtained using this technology by culturing BHK-21 in a 50L BIOSTAT® STR bioreactor. With the conditions stablished the reproductibility and the scale-up from 50L to 1000L can be easely performed.

REF-D032

COMPARISON OF BHK-21 CELL GROWTH ON MICROCARRIERS VS IN SUSPENSION AT 2L SCALE BOTH IN CONVENTIONAL BIOREACTOR AND SINGLE-USE BIOREACTOR (UNIVESSEL® SU)

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KEY WORDS:
BHK-21 CELL LINE / BIOREACTOR / SINGLE-USE

BACKGROUND AND NOVELTY:
BHK-21 cells are the most commonly used cells for vaccine production. Not all cell lines can be adapted to suspension growth. In general, anchorage-dependent (must be attached to a substrate to grow) cells will grow in suspension only with the use of microcarrier beads. However, some cell lines such as the BHK-21 which are not anchorage-dependent, can be adapted and variants that grow in suspension already exist. In recent years, the use of disposables in the pharmaceutical industry has increased extensively. The aim of this study is to evaluate the influence of a single use bioreactor on the final cell production of BHK-21 cells when they are growing with microcarriers or in suspension which can do an impact on the final product quality. Cultivations on conventional 2L-bioreactors were compared with results obtained from 2L single use bioreactor (UniVessel® SU).

EXPERIMENTAL APPROACH:
Two BHK-21 cell lines were used:
1.BHK-21 clone C3 as an ancorache dependent cell line
2.SBHK cells adapted to growth in suspension.
Both cell lines were cultivated in Glasgow medium supplemented with 10% (v/v) fetal bovine serum.
The growth using two different bioreactors was analyzed:
1.Conventional glass vessel of 2L using a BIOSTAT® Bplus as a control unit
2.The UniVessel® SU a s a Single use bioreactor, using a BIOSTAT® Bplus as a control unit
Parameters as pH, tª, stirring speed, aeration rate and viable cell number were analyzed.

RESULTS AND DISCUSSION:
Optimization and characterization of BHK-21 cells culture processes in both bioreactors was done.
BHK-21 attached and grew efficiently on microcarriers and the SBHK cells reach much higher densities inside the bioreactor than in a spinner flask. This is most likely due to the increased oxygenation inside the bioreactor, as well as the ability to control pH.
Higher yields were obtaining using SBHK cells.
The results were compared and the feasibility of transferring the BHK growth from a conventional bioreactor to single-use bioreactor has been demonstrated.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-D033

OPTIMIZATION OF THE RABIES VIRUS GLYCOPROTEIN EXPRESSION IN MAMMALIAN CELLS CULTIVATED IN SERUM FREE MEDIUM USING A RECOMBINANT SEMLIKI FOREST VIRUS

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KEY WORDS:
SERUM FREE MEDIUM / SEMLIKI FOREST VIRUS / RABIES GLYCOPROTEIN

BACKGROUND AND NOVELTY:
The present study aims to optimize the Semliki Forest Virus (SFV) system. For the expression of rabies virus glycoprotein (RVGP), recognized as an antigen capable of conferring immune response against rabies: 1. establish an efficient protocol for in vitro transcription and transfection of RNA to produce recombinant SFV (SFV-RVGP); 2. to determine the best conditions for cell culture and viral infection for the heterologous protein expression; 3. to analyze the RVGP expression in cells infected by SFV-RVGP by ELISA.

EXPERIMENTAL APPROACH:
Two different plasmids were used: an expression plasmid containing SFV genes coding for nonstructural proteins and the RVGP gene, and a helper plasmid containing SFV genes coding for structural proteins. Padronization of in vitro transcription were performed and RNAs were co-transfected in BHK-21 cells cultivated in different serum free medium, for generation of SFV-RVGP. They were then activated with alpha-chemotripsin and used to infect BHK-21 cells (also cultivated in different serum free medium). The cell inoculum was of 7x1E5 cells/well with a working volume of 2 mL.

RESULTS AND DISCUSSION:
The amount of protein produced was of 3.7 mcg/1E7 cells and preliminary results indicated better expressions in serum free medium. The production of SFV-RVGP virus, followed by the RVGP, 48 h after transfection and infection, respectively were higher when compared to that obtained after 24 hours.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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PROTEOMIC MONITORING ALONG THE ADAPTATION OF MDCK CELLS FROM ADHERENT TO SUSPENSION GROWTH WITH RESPECT TO INFLUENZA VIRUS VACCINE PRODUCTION

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KEY WORDS:
CELL LINE ADAPTATION / CELL LINE DEVELOPMENT / SUSPENSION CELL / QUANTITATIVE PROTEOMICS

BACKGROUND AND NOVELTY:
Under serum-free conditions adherent cells like Madin-Darby canine kidney (MDCK) cells attach poorly to the surface. With some patience and experience they can, however, slowly be adapted to growth in suspension. To better understand the impact of such a modification on cell culture-based influenza vaccine manufacturing [1], quantitative analysis on proteome level was performed. The focus was specifically on: What proteomic changes can be observed in independently repeated adaptations?

EXPERIMENTAL APPROACH:
Two independent two-step adaptations, phase 1: serum reduction in culture flasks, phase 2: generation of a homogeneous culture in spinner flask, were performed. Additionally, the adherent cell line and one of its resulting suspension cell lines (MDCK.SUS2) were used as substrate for influenza virus replication and for further proteome studies. Accordingly, 2-D DIGE comparisons were performed at several time points during adaptation, at different growth phases, and along influenza virus propagation. Regulated proteins were identified by mass spectrometry and their function elucidated by comparisons with homologous proteins.

RESULTS AND DISCUSSION:
For both independent adaptations phase 1 showed the strongest impact on the cellular proteome demonstrating importance and major impact of serum reduction (> 100 protein spots down-regulated, > 200 protein spots up-regulated). In large parts, both adaptations showed similar protein expression changes - mainly for proteins involved in metabolic pathways and cytoskeletal structures. However, also differences between both adaptations could be detected suggesting a more or less flexible response for cell line adaptation to growth in suspension. Despite the detected specific proteomic changes during the two adaptations, the proteomes of the adherent and the suspension cell line chosen for further studies did still show basic similarities. This could explain correlations regarding induced host cell responses and HA-titers of both cell lines after influenza infection.

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SHEFF-VAX SUPPLEMENT SIGNIFICANTLY REDUCES THE AMOUNT OF FBS REQUIRED FOR CULTURE OF VERO, MRC5 AND BHK21 CELLS

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KEY WORDS:
VACCINE MANUFACTURING / REDUCING FBS / MEDIA SUPPLEMENTATION / BHK21 CELLS / VERO CELLS

BACKGROUND AND NOVELTY:
The use of FBS has been indispensable for the growth of traditional mammalian cell lines including adherent and suspension cells. However, FBS has many disadvantages such as batch to batch variability, ethical concerns and most importantly it introduces the risk of contamination of production environments with adventitious agents. Advances in cell and tissue culture techniques have led to the increased of usage mammalian cells in vaccine manufacturing. This has resulted in a desire to reduce dependence on FBS for growth and maintenance of cell cultures.

EXPERIMENTAL APPROACH:
Classical basal medium's DMEM or GMEM were supplemented with Kerry's Sheff-Vax supplements in an effort to reduce the concentration of FBS in the growth medium of the three industrially relevant cell lines Vero, MRC5 and BHK21. The cells were initially maintained in classical basal media and 10% FBS. The cell lines were sequentially adapted to low FBS levels by gradual reduction of FBS in the presence of various concentrations of Sheff-Vax supplement. The optimal concentration of Sheff-Vax required depended on the cell line as well as the medium conditions. Images of the adherent cell lines Vero and MRC-5 were captured at different stages to check for abnormal morphological changes. Once the FBS concentration was reduced to 1.5%, Sheffield rInsulin ACF was required at 10 mg/L to support growth. Adherent VERO cells and suspension BHK21 cells were maintained in medium with 1% FBS supplemented with Sheff-Vax. Both cell lines showed comparable growth and viability to cells maintained in medium with just 10% FBS. The adherent MRC5 cells were maintained in medium with 1.5% FBS supplemented with Sheff-Vax and growth and viability was comparable to the cells maintained in 5% FBS. No morphological changes were observed in either adherent cell line.

RESULTS AND DISCUSSION:
The study shows that classical media supplemented with Sheff-Vax supplements can significantly reduce FBS dependency for all three of these industrially important cell lines.

REF-D036

EFFICACY AND TOXICITY OF THREE COMMERCIALLY AVAILABLE RECOMBINANT TRYPSINS VERSUS PORCINE TRYPSIN IN SIX DIFFERENT CELL LINES

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KEY WORDS:
TRYPSIN / VACCINE PRODUCTION / MEDIA SUPPLEMENT / VERO / MRC-5

BACKGROUND AND NOVELTY:
Trypsin is a serine protease found in the digestive system of most mammals. In cell culture, trypsin derived from porcine pancreases has historically been utilized. Trypsin is used to resuspend adherent cells attached to cell culture flasks. It is also used in primary cell culture/ cell isolation techniques to break down clumps of tissue into singular cells. Trypsin also has an application in influenza vaccine production in MDCK cell lines, where it increases virus infectivity by cleaving haemagluttinin. Although trypsin for a variety of cell culture applications has historically been extracted from porcine pancreas, the trend toward animal component free (ACF) media ingredients has led to an increasing interest in recombinant trypsin (rTrypsin). In this study, the performance of three commercially available rTrypsins was assessed alongside a native trypsin (animal derived) in 6 different cell lines.

EXPERIMENTAL APPROACH:
MRC-5, L929, C2C12, LLCPK1, MDCK and VERO cells were cultivated in FBS supplemented media for 5 successive passages. The cells were detached from flasks at each passage using a native trypsin as a control and 3 rTrypsins, TrypLE (Invitrogen), TrypZEAN (SIGMA) and r-Trypsin (Sheffield). The time for monolayer detachment was recorded and the action of each trypsin was filmed. The population doubling length (PDL) of each cell after passage with each trypsin was also calculated to see if any cumulative toxicity occurred.

RESULTS AND DISCUSSION:
The study shows, in a broad range of cell lines, that recombinant trypsin is an alternative to native trypsin in terms of effectiveness and ease of use. The study also shows that some recombinant trypsins can have toxic effects on the cells over time.

REF-D037

COMPARISON OF HEK293 AND SF9 CELL SUBSTRATES FOR THE PRODUCTION OF HEPATITIS C VACCINE CANDIDATES

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KEY WORDS:
VACCINES / RETROVIRUS LIKE PARTICLES / HEPATITIS C / SF9 CELLS / HEK293 CELLS

BACKGROUND AND NOVELTY:
Hepatitis C virus (HCV) infection is a major public health problem, causing more than 350.000 deaths every year, according to the World Health Organization. Currently, there is no Hepatitis C vaccine and the standard treatment for acute HCV infections has several limitations, including its low efficacy. There is thus a clear need for the development of a vaccine with both preventive and therapeutic roles. Our aim is to study different hosts for the production of retrovirus like particles as potential Hepatitis C vaccines. The insect cell production platform has attractive features for recombinant protein production, particularly its potential to achieve high productivities, coupled with easy scale-up. In this work we aim at evaluating the potential of Sf9 cells for the production of HCV pseudotyped retroVLP and compare its performance to that of the traditional cell substrate for retrovirus production, the HEK293 cells.

EXPERIMENTAL APPROACH:
Retrovirus-based particles were produced in Sf9 cells by the transfection with plasmids carrying Moloney murine leukemia virus (MLV) Gag-Pro and HCV envelope proteins (E1 and E2) under the control of OpIE2 promoters. For retroVLP-HCV production in human cells, a MLV Gag-Pol expressing HEK293 cell line was stably transfected with HCV-E1/E2. The particles produced in the different hosts were purified from culture supernatants and characterized.

RESULTS AND DISCUSSION:
MLV Gag and HCV-E1/E2 expression was readily detected in both Sf9 and HEK293 cells by western-blot analysis. Both production systems yielded over 1x10E9 pp/mL. The size distribution profile obtained by nanoparticle tracking analysis was similar for VLP produced in HEK293 and Sf9 cells. Determination of HCV-E1E2 glycosylation profile, ELISA analysis of envelope incorporation and electron microscopy studies are ongoing to further characterize retroVLP HCV. Our data shows that insect cells are promising as an alternative substrate to produce retrovirus based biopharmaceuticals.

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THE COMBINED USE OF PLATINUM NANOPARTICLES AND HYDROGEN MOLECULES INDUCES CASPASE-DEPENDENT APOPTOSIS

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KEY WORDS:
APOPTOSIS / NANOPARTICLES / HYDROGEN

BACKGROUND AND NOVELTY:
We previously reported that electrochemically reduced water (ERW), produced near the cathode by electrolysis, has reductive activity. We also revealed that ERW contains a small amount of Pt nanoparticles (Pt NPs) derived from Pt-coated titanium electrodes in addition to high concentration of dissolved molecular hydrogen by in vitro assay. Pt NPs exhibit multifunctional reactive oxygen species (ROS)-scavenging activity and catalytic action converting molecular hydrogen to atomic hydrogen(1). In this study we intend to investigate apoptosis pathway in HL60 cells activated by atomic hydrogen produced by catalytic action of Pt NPs in the presence of hydrogen molecules.

EXPERIMENTAL APPROACH:
Human promyelocytic leukaemia HL60 cells were cultured in RPMI 1640 medium supplemented with 10% fatal bovine serum, 2.0 mM l-glutamine, 100 U/ml penicillin and 100 U/ml streptomycin under an atmosphere of 5% Carbon dioxide. The cells were then incubated under an atmosphere of 75% hydrogen/ 20% oxygen/ 5% carbon dioxide, 75% helium/ 20% oxygen/ 5% carbon dioxide or 75% nitrogen/ 20% oxygen/ 5% carbon dioxide for 12 - 48 h after incubated with Pt NPs for 2 h. The cell growth was determined by counting cell numbers. Apoptosis pathway of HL60 cells was investigated by Sub G-1 assay.

RESULTS AND DISCUSSION:
The cell growth was strongly suppressed by the combined treatment of Pt NPs and hydrogen, but not by the sole treatment of Pt NPs or hydrogen. Analysis of cell cycle and caspase-3 activity suggested that the combined use of Pt NPs and hydrogen induced apoptosis in HL60 cells. The apoptosis was strongly inhibited by caspase-12 inhibitor. These results suggest that atomic hydrogen from hydrogen molecule by catalytic action of Pt NPs induces caspase-12-dependent apoptosis in HL60 cells.

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IMPACT OF A HIGH CELL DENSITY PERFUSION CELL CULTURE PROCESS ON ALTERNATING TANGENTIAL FLOW (ATF) PERFORMANCE: ATF PERFORMANCE ENHANCEMENT VIA BIOMASS REMOVAL LEADING TO INCREASED PRODUCTIVITY

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KEY WORDS:
ATF / BIOMASS REMOVAL / HIGH CELL DENSITY PERFUSION PROCESS

BACKGROUND AND NOVELTY:
Over the past decade, Cell Lines have achieved High yields by increasing specific productivity of individual cells and/or increasing the level of viable cell density available to the process. A cell culture process transferred to Janssen Biologics Ireland (JBIL) was an example of such a process i.e., high viable cell density that maximizes productivity, ensures product quality and reduces cost of goods. The cell culture process utilized an NS0 cell line for the production of a monoclonal antibody via a perfusion process. During process development/manufacturing, scale up of the cell culture process was initiated . The scale up evaluation was driven by KLa and Mixing requirements and other scale dependent process parameters to ensure adequate process control. A 50 day mammalian cell culture process, viable cell densities of greater than 35.0 x 106 VC/mL and the need for multiple ATF change outs were anticipated.

EXPERIMENTAL APPROACH:
During the large scale manufacture of the cell culture process, the high viable cell density and low culture viability lead to increased total cell density. From culture day 26 onwards, this high total cell density caused a deterioration of ATF performance manifesting initially as weight displacement excursions, leading to eventual operational failure of the ATF units. On cell culture day 37, process was terminated due to ATF operational failure.

RESULTS AND DISCUSSION:
Biomass removal strategy was successfully implemented for the process with no impact on Viable cell density, yield and product quality, a stabilization of the total cell density, enhanced ATF-10 performance, and achievement of the target 50 day cell culture process. The complete product quality and cell culture process data set will be described and discussed.

REF-D040

ENHANCEMENT MECHANISM OF ANTIOXIDANT ENZYME GENE EXPRESSION BY HYDROGEN MOLECULES

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KEY WORDS:
HYDROGEN / ANTIOXIDANT ENZYMES / NRF2 / OXIDATIVE STRESS / REDOX REGULATION

BACKGROUND AND NOVELTY:
Redox regulation system protects our body from oxidative stress-injury and keeps redox homeostasis. Hydrogen bacteria in intestine produces large quantities of hydrogen molecules and hydrogen gas is abundant one following to oxygen and carbon dioxide gas in blood. Recently, a number of studies demonstrated that hydrogen molecules improve oxidative stress-related diseases such as ischemia-reperfusion injury, glaucoma, Parkinson's disease and atherosclerosis in animal models. It is supposed from these positive results that hydrogen molecules can reduce oxidative stress, however, the mechanism of action of hydrogen molecules on oxidative stress has not been clarified yet. We hypothesized that intracellular redox regulation system might be activated by hydrogen molecules to enhance the antioxidative abilities of cells and animals. Thus, we examined the effect of hydrogen molecules in cultured cells damaged by hydrogen peroxide on the expression of antioxidant enzyme genes, Nrf2 gene and Nrf2 protein.

EXPERIMENTAL APPROACH:
A human fibrosarcoma cell line HT1080 was cultured in a gas incubator under a H2-rich atmosphere of 75%H2/20%O2/5%CO2 or a N2-rich atmosphere (75%N2/20%O2/5% CO2). The quantity of intracellular H2O2 was determined by In Cell Analyzer using BES-H2O2. Real-time PCR was done to examine the expression of antioxidant enzyme genes. The quantity of intracellular Nrf2 protein was determined by In Cell Analyzer using anti-Nrf2 antibody.

RESULTS AND DISCUSSION:
The quantity of intracellular H2O2 increased by hydrogen peroxide treatment was significantly decreased by pretreatment of H2. H2 enhanced the expression of catalase, glultathione peroxidase, Cu/Zn-superoxide dismutase, Nrf2 genes and Nrf2 protein. In conclusion, it was suggested that H2 induced the expression level of the antioxidant enzyme genes like catalase and glutathione peroxidase by increasing the expression level of the Nrf2 protein and alleviating the quantity of intracellular H2O2 in HT1080 cells.

REF-D041

ANALYSIS OF THE MECHANISM OF PLATINUM NANOPARTICLES AS REDOX REGULATION FACTORS

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KEY WORDS:
NANOPARTICLE / PLATINUM / ROS / REDOX / NRF2

BACKGROUND AND NOVELTY:
So far, most of studies on nanometer-sized metal particles have focused on biological safety and potential hazards. However, anti-oxidative activity of metal nanoparticles (NPs) attracts much attention, recently. Platinum nanoparticles (Pt NPs) are one of the most important metals in nanotechnology because Pt NPs have negative surface potential from negative charges and are stably suspended from an electric repulsion between the same charged particles(1). We previously reported that Pt NPs of 2-3 nm sizes scavenged reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide and hydroxyl radical in vitro(2). Here, we report the cytotoxicity and antioxidative activity of Pt NPs of 2-3 nm sizes.

EXPERIMENTAL APPROACH:
Pt NPs were synthesized by a modified citrate reduction method of Hydrogen hexachloro-patinate(VI). Particle size and concentrations were determined by a transmittance electron microscope and ICP-MS, respectively. Rat myoblast L6 cells were pre-cultured for 24 h in a DMEM with a given amount of Pt NPs and cell viability was determined by WST-1 and LDH assays. Intracellular glutathione level was measured by spectrofluorometry and ROS level by imaging cytometry. The gene expressions of anti-oxidative enzyme-related genes were investigated using real-time PCR.

RESULTS AND DISCUSSION:
Cytotoxicity of Pt NPs of 2-3 nm sizes was not observed at a concentration below 10 ppm. Intracellular glutathione concentration was increased and the amount of intracellular ROS was decreased significantly by the Pt NPs treatment. The real-time PCR analysis revealed that the gene expressions of glutathione reductase, glutathione peroxidase, heme oxygenase-1 and manganese superoxide dismutase were increased by the Pt NPs treatment. These results suggest that Pt NPs of 2-3 nm sizes have little cytotoxity and are expected as redox regulation factors for suppression of various ROS-related diseases.

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FUCOIDAN EXTRACT ENHANCES THE ANTI-CANCER ACTIVITY OF CHEMOTHERAPEUTIC AGENTS IN BREAST CANCER CELLS

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KEY WORDS:
FUCOIDAN / CHEMOTHERAPEUTIC AGENT / BREAST CANCER CELLS / APOPTOSIS / REACTIVE OXYGEN SPECIES

BACKGROUND AND NOVELTY:

Fucoidan, a fucose-rich polysaccharide isolated from brown alga, is currently under investigation as a new anti-cancer compound (1). In the present study, fucoidan extract (FE) from Cladosiphon navae-caledoniae Kylin was prepared by enzymatic digestion. We investigated whether a combination of FE with chemotherapeutic agents had the potential to improve the therapeutic efficacy of cancer treatment.

EXPERIMENTAL APPROACH:

Estrogen receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells were cultured in DME medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO2 at 37 °C. The abalone glycosidase-digested fucoidan extract (FE) was obtained from Daiichi Sangyo Corporation (Osaka, Japan). The cells were treated with FE and chemotherapeutic agents like cisplatin, tamoxifen or paclitaxel. The cell growth was determined by MTT assay. Apoptosis was evaluated using annexin V binding assay and flow cytometry analysis. Signaling proteins were analyzed by western blot. Intracellular reactive oxygen species (ROS) were determined using DCFH-DA and determined using IN Cell Analyzer 1000. The reduced glutathione (GSH) concentration was measured by the GSH assay kit.

RESULTS AND DISCUSSION:

The combined use of FE and chemotherapeutic agents significantly induced cell growth inhibition, apoptosis, as well as cell cycle modifications in both MDA-MB-231 and MCF-7 cells. FE enhanced apoptosis in cancer cells that responded to treatment with three chemotherapeutic drugs with downregulation of the anti-apoptotic proteins Bcl-xL and Mcl-1. The combination treatments led to an obvious decrease in the phosphorylation of ERK and Akt in MDA-MB-231 cells, but increased the phosphorylation of ERK in MCF-7 cells. In addition, we observed that combination treatments enhanced intracellular ROS levels and GSH levels in breast cancer cells, suggesting that induction of oxidative stress was an important event in the cell death induced by the combination treatments.

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DOWNREGULATION OF THE MITF GENE EXPRESSION AND ACTIVATION OF ERK PATHWAY IN HUMAN AND MURINE MELANOMA CELL LINES –POSSIBLE MECHANISM FOR THE ANTIMELANOMA EFFECT OF DAPHNANE DITERPENE HIRSEIN B

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KEY WORDS:
MELANOMA / MITF (MICROPHTHALMIA-ASSOCIATED TRANSCRIPTION FACTOR) / ERK / DAPHNANE DITERPENE

BACKGROUND AND NOVELTY:

Melanoma is one of the main causes of death of skin cancer patients in Europe, Australia, and the United States (http://www.skincancer.org/skin-cancer-information/melanoma). Melanoma has been the focus of most research groups but the signal transduction pathways that regulate its development and progression is yet unknown (Jiang et al. 1994). As the incidence of melanoma worldwide continue to increase, alternative approaches that target the tumor, beyond the usual radiation therapy and chemotherapy are being considered.
The role of microphthalmia-associated transcription factor (MITF) in melanocyte development, function, and survival and as an amplified oncogene in melanoma cells have been reported (Levy et al., 2006). The reduction of MITF activity, has been suggested to sensitize melanoma cells to chemotherapeutic agents (Garraway et al., 2005). We have previously reported that hirsein B (HB) can downregulate the expression of Mitf in B16 cells (Villareal et al., 2010) but the effect of HB on the MITF expression and on the differentiation of aggressive melanoma cells is not yet determined.

EXPERIMENTAL APPROACH:

We quantified the MITF gene using real-time PCR and the activation of ERK1/2 using western blot in HB-treated human melanoma cells SK-MEL-5 and SK-MEL-28. Furthermore, to determine the effect of HB on the activation of MITF protein, western blotting was performed. The effect on the cycling of the cells was done using flow cytometry. In addition, changes in the cell morphology was observed by staining the cells with rhodamine phalloidin.

RESULTS AND DISCUSSION:

Results show that HB caused a significant decrease in the expression of the MITF gene in SK-MEL-5 cells but not in SK-MEL-28 cells. However, even though HB failed to downregulate the MITF gene expression in SK-MEL-28, the increase in the expression of activated ERK1/2 will lead to MITF protein degradation. Determination of the expression of the MITF protein confirmed this results. Both SK-MEL-5 and SK-MEL-28 exhibited changes in the cell morphology characterized by an increase in cell dendricity, similar to what was observed in B16 cells (Villareal et al., 2010). Murine and melanoma cells have been reported to exhibit differences as far as the mechanisms regulating functions such as cell proliferation, cell cycle, among others. Results of cell cycle analysis revealed that HB had the same effect on both B16 and human melanoma cells, hinting at the possibility that HB does not have significant effect on the cycling of the cells. The observed increase in cells' dendritic extensions suggests an actin cytoskeleton reorganization, which supports the results of previous study wherein an increase in the expression of the MAPKAPK3 gene in HB-treated B16 cells was observed. The results of this study highlights the potential of daphnane diterpene HB as a potential therapeutic agent that can be used to decrease the MITF activity in order to sensitize melanoma cells to chemotherapeutic agents against melanoma.

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NOVEL HUMAN KIDNEY EPITHELIAL CELL LINES IN MEDICAL BIOTECHNOLOGY

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KEY WORDS:
HUMAN KIDNEY EPITHELIAL CELL LINES / TOXICOLOGY / PRODUCTION SYSTEM / HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS

BACKGROUND AND NOVELTY:
Human cell cultures are of ever increasing importance in medical biotechnology, where they find applications as model systems, producers as well as products themselves. The development of new cell lines with defined characteristics is therefore of high interest for toxicology, production of biopharmaceuticals or as product itself.

EXPERIMENTAL APPROACH:
Human proximal tubular epithelial cells were isolated from kidney tissue biopsies as well as urine sediments. In order to overcome the restricted replicative life span of the cells grown in vitro and thus the limitation of the use of the cells, we introduced either viral proteins or the catalytic subunit of human telomerase. Selected cell clones displaying an extension of the cellular life span were extensively characterised for cell type specific markers and functions as well as their potential as well standardized model system or as production system.

RESULTS AND DISCUSSION:
We demonstrate the establishment of continuously growing, highly differentiated human proximal tubular epithelial cell lines that show a high potential as novel hosts for production of high quality biopharmaceuticals as well as in-vitro toxicity model systems.

ASSESSMENT OF TROGLITAZONE INDUCED LIVER TOXICITY IN A DYNAMICALLY PERFUSED TWO-ORGAN MICRO-BIOREACTOR SYSTEM

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KEY WORDS:
MULTI-ORGAN-CHIP / SUBSTANCE TESTING / TISSUE ENGINEERING / MICRO-BIOREACTORS / ORGANOIDS

BACKGROUND AND NOVELTY:
The ever-growing amount of new substances released to the market and the limited predictability of current in vitro test systems has led to an ample need for new substance testing solutions. Many drugs like troglitazone, that had to be removed from the market due to drug induced liver injury, show their toxic potential only after chronic long term exposure. But for long-term multiple dosing experiments, a controlled microenvironment is pivotal, as even minor alterations in extracellular conditions may greatly influence the cell physiology. Within our research program, we focused on the generation of a micro-engineered bioreactor, which can be dynamically perfused by an on-chip pump and combines at least two culture spaces for multi-organ applications. This circulatory systems better mimics the in vivo conditions of primary cell cultures and assures steadier, more quantifiable extracellular signaling to the cells.

EXPERIMENTAL APPROACH:
A dynamically perfused co-culture of human skin punch biopsies and liver aggregates was performed for a period of 7 to 14 days inside the micro-bioreactor. These cultures were treated with troglitazone at different concentrations throughout the culture period. The production of albumin, urea and lactate, as well as the consumption of glucose and the release of LDH into the culture medium was analyzed daily. Live-dead cell count, as well as the expression of selected markers were analyzed by immunofluorescence and real-time PCR.

RESULTS AND DISCUSSION:
It could be shown, that this micro-bioreactor system is capable of supporting long-term co-cultures of skin biopsies and human liver equivalents. The liver toxic effect of troglitazone could be successfully modeled in this system. An increase in the release of LDH to the culture medium could be observed during the experiment, as well as a markedly increase in dead liver cell count at troglitazone treated cultures.

REF-D046

FUNCTIONAL IMMORTALIZED ENDOTHELIAL CELL LINES FOR DEVELOPMENT OF BIOASSAYS

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KEY WORDS:
PHYSIOLOGICAL RELEVANCE / IMMORTALIZATION / BIOASSAYS / ENDOTHELIAL CELLS

BACKGROUND AND NOVELTY:
Endothelial cells are specialized cells that line blood vessels. Dysfunction of the endothelium is observed in severe diseases like diabetes, hypertension, cancer and coronary artery disease. For identification of relevant targets/drug candidates cellular test systems are highly desirable that closely reflect the in vivo properties. We recently described such functional immortalized endothelial cells that were derived from the umbilical cord. In addition, to the physiological relevance these novel test systems should also be robust in order to facilitate the development of the required bioassays.

EXPERIMENTAL APPROACH:
We used a functional immortalized human endothelial cell line (CI-SCREEN HUVEC) that we previously established. The key feature of this cell system is that it combines the positive advantage of cell lines – the unlimited cell supply – with the advantage of primary cells – the physiological relevance. This cell line was used to establish robust bioassays.

RESULTS AND DISCUSSION:
The CI-SCREEN HUVEC cell system is a novel immortalized endothelial cell line that can be maintained in culture for more than 120 population doublings. Importantly, the cellular phenotype of this cell line is stable throughout the cultivation period and is comparable to primary HUVEC. This cell system formed the basis for the development of robust bioassays which help to investigate angiogenesis. In a first proof of concept study the effect of known inducers of angiogenesis (like e.g. VEGF, FGF) was investigated. In addition, also the opposing effect of known anti-angiogenic molecules was monitored.
Our study demonstrates the utility of the CI-SCREEN HUVEC as this cell system enables the development of robust and reliable bioassays.

REF-D047

SEARCHING FOR COMPOUNDS THAT MODULATE TYPE I INTERFERON ACTIVITY BY SCREENING LIBRARIES WITH NEW REPORTER CELL LINES

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KEY WORDS:
REPORTER GENE ASSAYS / EGFP / MX PROMOTER / IFN-ALPHA / IFN-BETA

BACKGROUND AND NOVELTY:
IFNs have dual clinical effects depending on their use as pharmaceuticals (IFN-alpha or IFN-beta) or their special feature of being self-produced by humans in some autoimmune diseases (IFN-alpha). Thus, increasing their therapeutic efficiency in the first situation or decreasing the side effects in the second one involve high clinical value. In this sense, it is interesting to find molecules which can modulate the IFN´s activity.

EXPERIMENTAL APPROACH:
We carried out a simple, fast and robust reporter assay to identify IFN activity modulator compounds. We analyzed lots of them simultaneously giving reliable and reproducible results employing Mx2/eGFP modified-A549, -HeLa, -HEp2 and -WISH reporter cell lines previously developed in our lab. In these cell lines the enhanced green fluorescence protein (eGFP) gene is driven by the specific type I IFNs inducible Mx2 promoter. The eGFP percentage obtained after type I IFN addition is directly correlated with the cytokine in the sample. We used the reporter cell lines to analyze a complete natural library of 176 compounds and 288 from a synthetic library of 2,500 compounds provided by the Helmholtz Zentrum für Infektionsforschung, Braunschweig, Germany.

RESULTS AND DISCUSSION:
We isolated 21 inhibitory compounds and 1 enhancer compound. All of them were characterized according to their cytotoxicity, effective doses, antiviral and anti-proliferative activity, residual and reversible effect, and their action on cell cycle and apoptosis. Along this screening, we could easily discriminate positive from negative compounds. The Z factor value, commonly used in high throughput screening, was always higher than 0.8, reflecting the excellent quality of the bioassay. This search for compounds which can improve or block type I IFN´s activity shows a big potential in view of their therapeutic implications.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
We are very grateful to Mario Köster, Hansjörg Hauser, Dieter Kaufmann and Florenz Sasse for being so kind in providing us the compounds libraries and for the exhaustive help during this work.

A MECHANISTIC DISSECTION OF POLYETHYLENIMINE MEDIATED TRANSFECTION IN CHO CELLS

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KEY WORDS:
POLYETHYLENIMINE / TRANSIENT GENE EXPRESSION / TRANSIENT TRANSFECTION

BACKGROUND AND NOVELTY:
Polyethylenimine (PEI) has been used as a gene delivery vehicle for a range of applications; it holds huge promise for gene therapy and has been used for over a decade in large scale transfection of mammalian cells for therapeutic recombinant protein production by transient gene expression (TGE)1. Despite the widespread applications of PEI, little is known about the mechanism of transfection at the sub-cellular level, with many conflicting hypothesise proposed. We present data exploring the molecular interactions between PEI-DNA polyplexes and the surface of CHO-S cells, using a transfection protocol optimized by Design of Experiments methodology2.

EXPERIMENTAL APPROACH:
Suspension adapted CHO-S cells were transfected in supplemented CD-CHO media with 25kDa linear PEI and plasmid DNA. A microscale 24 well plate platform was used. Polyplex-cell surface binding experiments were performed at hypothermic conditions (4°C), using fluoro labelled plasmid DNA, flow cytometry and confocal microscopy.

RESULTS AND DISCUSSION:
Polyplex-cell surface binding and subsequent internalization were found to be both rapid, saturable processess; over 8 hours post transfection, cell surface attached polyplex was found to remain at an approximatley constant level. Cell surface heparan sulphate proteoglycans (HSPGs) were found to deplete on the cell surface following transfection (>80% reduction 30 minutes post transfection) and remain depleted, in contrast to rapid regeneration of HSPGs following enzymatic cleavage (>70% at 4 hours post transfection). Enzymatic cleavage of HSPGs from the cell surface (>90% knock down, as validated by immunostaining) reduced transient SEAP production by ~20% at 24 hours post transfection. Chemical sequestration of cholesterol from the cell surface reduced transient SEAP production in a concentration dependent manner, at higher concentrations eliminating transfection. Taken together, we suggest that polyplex-cell surface binding is mediated by interactions with HSPG associated lipid rafts.

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PRODUCTION OF RFVIII USING A SKHEP STABLY TRANSFECTED CELLS IN CONTROLLED PERFUSION CULTURE

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KEY WORDS:
PERFUSION / RFVIII / CELL CULTURE

BACKGROUND AND NOVELTY:
Coagulation factor VIII is a complex glycoprotein that performs a critical role in the intrinsic blood coagulation pathway. Its deficiency is associated with the hemophilia, a bleeding disorder that affects 1 in 5.000 to 10.000 males. The production of the recombinant factor VIII (rFVIII) can only be performed by mammalian cells, due to its complexity. A SkHep cell line, stably transfected to express a B-deleted rFVIII, was used in this work. As serum-free medium adaptations were not successful, microcarriers systems were adopted. In order to overcome rFVIII high thermolability, a perfusion system was proposed.

EXPERIMENTAL APPROACH:
Cells were grown on 3 g.L-1 Cytodex 3 (GE) microcarriers in 1.5 L bubble free bioreactor, with an internal spinfilter, at pH of 7.4, 30% DO (air saturation), 37 ºC, employing a supplemented-serum medium DMEM. Three independent feed solutions (glucose, glutamine and basal medium) were applied in order to maintain the glucose and glutamine concentrations at 1 g.L-1 and 0.3 g.L-1, respectively. The perfusion rate was set at 1 vvd.

RESULTS AND DISCUSSION:
After 143h of batch cultivation, system was set at perfusion operation mode. A transient phase of 250h was observed, during which cells grow linearly up to 1,31E+06 ± 4,37E+05 cel.mL-1, a value 10% higher than that attained in batch runs (previous data), probably due to glucose limitation overcoming.
A steady-state phase of approximately 100 h (4 residence time-RT) for viable cell, and 175 h (7 RT) for glucose, lactate and ammonium concentrations, were than observed. Concerning rFVIII concentration, a shorter steady state phase (55 h; 2.3 RT), and also less well-characterized (1,64 ± 0,76 UI.mL-1) were denoted. This result was considered promising in view of the possibility of reducing natural protein degradation in perfusion mode.

REF-D050

OPTIMIZATION OF UPCYTE® PROCESS ALLOWS FOR THE GENERATION OF PROLIFERATING HUMAN HEPATOCYTES WITH ENHANCED CYP ENZYME ACTIVITIES

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KEY WORDS:
UPCYTE® HEPATOCYTE / PHASE 1 AND 2 METABOLISM / CYP P450 / DRUG DEVELOPMENT

BACKGROUND AND NOVELTY:
Primary cultures of human hepatocytes are routinely used in drug development to evaluate metabolic fate, drug-drug interactions and drug toxicity. However, the supply of hepatocytes is limited by the low and sporadic availability of human liver tissue. We have developed a novel technique, i.e. the upcyte® process, which allows for the generation of human hepatocyte cultures with the ability to proliferate whilst maintaining differentiated functions.

EXPERIMENTAL APPROACH:
These cells, named upcyte® hepatocytes, are not immortalized since they grow anchorage-dependently, as demonstrated in soft agar assays, and express the differentiated marker gene, REIC (Reduced Expression in Immortalized Cells)/Dkk-3. Confluent cultures of upcyte® hepatocytes expressed key liver-specific molecules, such as HSA, CK8, CK18 and AAT. In addition, the cells produced urea and were able to store glycogen, measured using PAS staining. As a result of continuous optimization of our novel upcyte® technology, we were able to generate proliferating human hepatocytes which possess up to 50% of CYP enzyme activities compared to the original primary cells. CYP1A2, CYP2B6 and CYP3A4 were inducible; moreover, upcyte® hepatocytes predicted the in vivo induction potencies of known CYP3A4 inducers using the "relative induction score" prediction model. Placing cells into 3D culture increased basal CYP2B6 and CYP3A4 basal activities, expression of their respective regulatory nuclear receptors (CAR and PXR); as well as induction responses. Phase 2 activities (UGTs, SULTs and GSTs) were comparable to the activities of freshly isolated hepatocytes. Upcyte® hepatocytes were markedly more sensitive to the hepatotoxin, a-amanitin, than HepG2 cells, indicating functional OATP1B3 uptake. In addition, upcyte® hepatocytes were able to differentiate between hepatotoxic and non-hepatotoxic compounds.

RESULTS AND DISCUSSION:
In conclusion, upcyte® hepatocyte cultures have a differentiated phenotype and exhibit functional phase 1 and 2 activities. These data support the use of upcyte® hepatocytes for induction and cytotoxicity screening assays. Moreover, this technology allows for the generation of large batches of upcyte® hepatocytes (up to 12x109 cells per donor) enabling a reproducible and standardized experimental setting.

REF-D051

IN VITRO CYTOTOXICITY AND GENOTOXICITY TESTING USING UPCYTE® HEPATOCYTES

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KEY WORDS:
GENOTOXICITY / CYTOTOXICITY / UPCYTE® HEPATOCYTE / DRUG DEVELOPMENT

BACKGROUND AND NOVELTY:
We have developed a novel technique which causes primary human hepatocytes to proliferate whilst retaining an adult phenotype including functional phase 1 and 2 metabolism. In this study we evaluated the resulting "upcyte® hepatocytes" for their potential use in cyto- and genotoxicity testing.

EXPERIMENTAL APPROACH:
For the establishment of an upcyte® hepatocytes based genotoxicity test we adapted the conditions of the frequently used micronucleus test by incorporating the use of upcyte® hepatocytes. Conditions for upcyte® hepatocytes from a single donor (Donor 740) showed that a treatment duration of 96 h, without a recovery period was optimal for detecting both directly acting (e.g. mitomycin C) and metabolically activated genotoxins (cyclophosphamide), whilst true negative and "false" or "misleading" positive compounds were correctly identified as negative. The basal MN rate of upcyte® hepatocytes was dependant on the thawing time point, medium components and the design of the assay. The %MN in upcyte® hepatocytes treated with DMSO, cyclophosphamide and MMC was essentially unaffected by the growth stage (between a population doubling (PD) of 18 and 59). In conclusion, these data support the use of upcyte® hepatocytes in the MN test, since they were able to correctly identify known direct and metabolically activated genotoxicants, while misleading positives and true negative compounds resulted in negative outcomes.

RESULTS AND DISCUSSION:
The cytotoxicity of 31 compounds was measured using upcyte® hepatocytes derived from four donors to include effects due to donor variation. The compounds were classified as either severely, moderately or non-hepatotoxic. There was a very good intra- and inter-experimental reproducibility of the measurements. The cytotoxicity of the majority of compounds was donor-dependent - donor 653 was generally less susceptible to cytotoxicity than donors 422A and 10. The predictive capacity of the assay was generally good such that known non-hepatotoxicants were clearly negative and compounds that were associated with hepatotoxicity caused damage to the upcyte® hepatocytes.
In conclusion, these studies show that use of upcyte® hepatocytes which combine proliferation with long-term stable expression of adult hepatic phenotype enables the development of new in vitro liver cell.

CAP-T CELLS IN COMBINATION WITH A NEWLY DEVELOPED CHEMICALLY DEFINED CULTURE AND TRANSFECTION MEDIUM - A POWERFUL PLATFORM FOR TRANSIENT RECOMBINANT PROTEIN EXPRESSION

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KEY WORDS:
TRANSIENT TRANSFECTION / CAP-T CELLS / CHEMICAL DEFINED MEDIA

BACKGROUND AND NOVELTY:
The CAP system, based on immortalized human amniocytes was specifically designed for successful production of complex glycosylated proteins. CAP-T cells, which are derived from CAP cells by stably over-expressing the SV40 large T-antigen, are highly suitable for transient protein expression. They are characterized by good transfection capabilities, easy cultivation in serum free suspension and high viability. Like CAP cells, CAP-T cells produce proteins with human-like glycosylation pattern and are capable of attaching terminal sialic acid. In conclusion, CAP-T cells are a promising tool for production of complex mammalian proteins for drug discovery, high-throughput screening, assay development or early stage pre-clinical drug development. Unfortunately, until now an up-scaling to large volumes was complicated by the need of an extra centrifugation step during transfection, changing the media from a growth media to a transfection media.

EXPERIMENTAL APPROACH:
In collaboration with TeutoCell AG, we developed a chemically defined and animal component free CAP-T culture and transfection media, to allow for easy upscaling of CAP-T transfection.

RESULTS AND DISCUSSION:
The new media enables long term culturing of CAP-T cells to high cell densities with excellent cell viabilities. In addition, it supports direct transfection of CAP-T cells with a variety of transfection reagents without the need of an extra media change during transfection. Side-by-side comparison of transient protein expression of CAP-T cells cultured and transfected in the new medium and 239F cells cultured and transfected in Freestyle™ media revealed similar or even higher protein yields with excellent product quality.

A MODULAR FLOW-CHAMBER BIOREACTOR CONCEPT AS A TOOL FOR CONTINUOUS 3D-CULTURE

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KEY WORDS:
TISSUE CULTUE / BIOREACTOR / FLOW CHAMBER / ELECTRICAL STIMULATION

BACKGROUND AND NOVELTY:
New challenges in drug development and drug testing arise from regulatory requirements. Animal trials have to be replaced by cell culture assays, preferably by test systems with human material. Standard 2 D monolayer cultures are often unsatisfac-tory and therefore tissue-like 3D cultures are suggested as an alternative. Here two concepts, a multi-well flow-chamber bioreactor and a multi-fixed bed bioreactor system as a tool for continuous 3D-culture are presented. These reactor concepts have been implemented for several applications.

EXPERIMENTAL APPROACH:
Applications for modular flow-chamber bioreactor:
- Cultivation of cartilage-carrier-constructs
- Three-dimensional liver culture on porous carriers for drug testing
- Resorption of magnesium implants
- Electrical stimulation of cells

Here the mentioned examples as well as engineering aspects will be discussed.

RESULTS AND DISCUSSION:
Advantages of these reactor concepts can be seen in constant culture conditions, removal of toxic reaction products, higher cell densities, and improved metabolism. On the other engineering aspects such as flow conditions, mass transfer effects, space time distribution have to be considered.

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REF-D054

ENHANCED CELL ADHESION AND VIABILITY OF CHITOSAN FILMS ENZYMATICALLY MODIFIED WITH PHENOLIC COMPOUNDS

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KEY WORDS:
CHITOSAN DERIVATIVE FILM / ENZYMATIC FUNCTIONALISATION / HUVEC

BACKGROUND AND NOVELTY:
Chitosan is considered as one promising polymeric materials in different industries such in wound healing and tissue-engineering due to its biocompatibility, biodegradability and low toxicity. However, chitosan film alone showed slightly cell adhesion property due to its high hydrophilicity [1]. Several works investigated physicochemical properties of chemically modified chitosan. Recently, the use of oxidative enzymes such as laccases has been shown to represent a potential alternative for modification of chitosan [2,3]. The present work examines the HUVEC adhesion and growth on chitosan films enzymatically modified with ferulic acid (FA) and ethyl ferulate (EF) [3]

EXPERIMENTAL APPROACH:
Grafting of oxidation products onto chitosan was performed using laccase from Myceliophtora thermophyla [3]. Chitosan films were prepared in 24-well plates, dried for 3 days, neutralized with NaOH for an hour, washed with distilled water and sterilized with UV. Human Umbilical Vein Endothelial Cells (HUVEC) were cultivated in Endothelial Basal Medium. For experiments, wells with chitosan films were filled with 1ml of HUVEC cells at 1.105 cells/well for 3 days. The morphology of attached cells was observed using an optical microscope and the cell viability was determined using MTT assay.

RESULTS AND DISCUSSION:
Oxidized phenol grafting onto chitosan led to FA-colored chitosan and EF-colorless chitosan. This process also increased its hydrophobic properties and decreased free amino groups. Consequently, it improved protein adsorption properties of modified chitosan films. The morphology of cells on modified chitosan films indicated a well attachment and spread phenotype while they remained round on chitosan films. Cell viability was improved on modified chitosan films. It was dependant on the thickness of film and the quantity of oxidized phenols grafted. Finally, FA-/EF-chitosan films showed almost similar cell viability. These innovative biomaterials are good candidates for biomedical applications especially tissue engineering.

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REF-D055

PROLIFERATION OF PRIMARY HUMAN T LYMPHOCYTES INSIDE OF POLYELECTROLYTE CAPSULES

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KEY WORDS:
ENCAPSULATION / HUMAN T LYMPHOCYTES

BACKGROUND AND NOVELTY:
An important method for future applications in biotechnology and medical research is the encapsulation of mammalian cells. Toward this goal, we focused our work on the encapsulation of human T lymphocytes in polyelectrolytes capsules made of sodium cellulose sulfate (NaCS) with an outer membrane consisting of the polycation poly(diallyldimethyl)ammonium chloride (polyDADMAC). Based on the protection of cells from sheer forces, we observed in previous works using a human T lymphocytes cell line (Jurkat) and human primary T lymphocytes that high cell densities (up to 100 x 106 cells/mLcapsules for Jurkat, > 15 x 106 cells/mLcapsules primary cells) and viabilities can be reached. Moreover, neither the growth rate nor the development of T lymphocyte subpopulations was affected by the capsule material. Beside this, the capsules show long-term stability and the capsule material a good biocompatibility. Therefore, the behavior of primary T cells (mono-culture vs. co-culture) in such a context as well as a further investigation of the microenvironment within the capsules is an important issue and the resulting data might be of high importance for Tissue Engineering approaches.

EXPERIMENTAL APPROACH:
In the present study, human primary T lymphocytes and monocytes isolated from the blood of healthy donors were encapsulated and grown under static and dynamic conditions. The influence of the microenvironment inside polyelectrolyte capsules in terms of material properties and cell physiology is presented.

RESULTS AND DISCUSSION:
In order to investigate the positive role, on cell division, played by the microenvironment inside the capsules, the production level of some relevant cytokines for T lymphocytes development was determined. Moreover, the interplay between the secreted cytokines and the NaCS within the capsules and its putative influence on cell growth will be discussed.

MULTICELLULAR TUMOR SPHEROIDS IN MICROCAPSULES AS A NOVEL 3D IN VITRO MODEL IN TUMOR BIOLOGY

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KEY WORDS:
TUMOR SPHEROIDS / SMART MICROCAPSULES / PHOTODYNAMIC THERAPY / 3D IN VITRO MODEL / CHITOSAN

BACKGROUND AND NOVELTY:
Advantages of microencapsulation as a 3D growth system are chemically and spatially defined 3D network of extracellular matrix components, cell-to-cell and cell-to-matrix interactions governing differentiation, proliferation and cell function in vivo. The study is aimed at i) optimization of techniques for preparing microcapsules; ii) generation of multicellular tumor spheroids (MTS) by culturing tumor cells in the microcapsules; iii) study of anticancer treatment effects for both photodynamic therapy (PDT) and anticancer drug screening. The model allows to estimate drug doses or parameters for PDT in vitro before carrying out preclinical tests, and thereby reduce a number and costs of experiments with animals commonly used.

EXPERIMENTAL APPROACH:
To form MTS, tumor cell lines (mouse melanoma cells M3, human breast adenocarcinoma cells MCF-7, mouse myeloma Sp2/0 cells, human CCRF-CEM and CEM/Cl cell lines, HeLa) were encapsulated in polyelectrolyte microcapsules (200-600 μm), and cultivated for 3-4 weeks. Microcapsules were fabricated from alginate (polyanion) and various polycations, namely natural polymers (modified chitosan, DEAE-dextran etc) and novel smart co-polymers (e.g.chitosan-graft-polyvinyl alcohol copolymers) synthesized by a novel Solid-State Reactive Blending method. The copolymers were characterized by FTIR, GPC and elemental analysis.

RESULTS AND DISCUSSION:
MTS based MCF-7 cells were prepared and used to study effects of PDT. To study the effect of irradiation parameters on cell viability, 2 photosensitizers (PS), namely photosense and chlorine e6 were used. Phototoxicity of PS depended on PS concentration and light energy density in both monolayer culture (MLC) and MTS. Study of cell morphology in MLC and MTS before and after PDT revealed that light energy density increase within the range of 30-70 J/cm2 resulted in cell apoptosis. However, cell survival in MTS was much higher than this in the MLC. MTS were also used to test some antitumor therapeutics (methotrexate, doxorubicin and their derivatives). An enhanced cell resistance in MTS compared to MLC both for normal and Dox-resistant cells (MCF-7, MCF-7/DXR, respectively) were observed. MTS were also proposed to evaluate cytotoxicity not only of novel therapeutics but also nanosized drug delivery systems (liposomes, micelles, nanoparticles and nanoemulsions).

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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DEVELOPMENT OF 3D HUMAN INTESTINAL EQUIVALENTS FOR SUBSTANCE TESTING IN MICROLITER-SCALE ON A MULTI-ORGAN-CHIP

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KEY WORDS:
MULTI-ORGAN-CHIP / INTESTINAL EQUIVALENT / SUBSTANCE TESTING / DYNAMIC CULTURE / BIOLOGICAL, VASCULARIZED MATRIX

BACKGROUND AND NOVELTY:
Robust and reliable dynamic bioreactors for long term maintenance of various tissues at milliliter-scale on the basis of a biological, vascularized matrix (BioVaSc®) have been developed at the Fraunhofer IGB in Stuttgart, Germany. As an intestinal in vitro equivalent, seeding of the matrix with CaCo-2 cells yielded in the self-assembly of a microenvironment with the typical histological appearance of villi-like structure and morphology (Pusch et al. Biomaterials 2011). We modified this matrix (BioVaSc®) – cell (CaCo-2) system to some extent with the aim to develop 3D intestinal equivalents for substance testing in microliter-scale on a multi-organ-chip.

EXPERIMENTAL APPROACH:
Jejunal segments of rats with the corresponding vascular system were explanted, decellularized, reseeded with CaCo-2 cells and integrated in a perfused multi-organ-chip device. Process parameters, such as nutrient perfusion rate and culture time, have been optimized to qualify the system for repeated dose testing of orally administered drug candidates. Daily medium samples have been analyzed to monitor metabolic activity and the absorption properties of the intestinal equivalent. Immunhistostaining of cryo-preserved tissue slices have been analyzed to compare self assembled organoid tissue structures with their corresponding in vivo counterparts.

RESULTS AND DISCUSSION:
Evidences are provided for the use of the system for reliable evaluation of absorption properties of drugs at different dosages over long periods. Further improvements of the system, e.g. by seeding the matrix with primary intestinal cells, are planned.

REF-D058

CELLULAR TOOLS FOR BIOSIMILAR MONOCLONAL ANTIBODY ANALYSIS

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KEY WORDS:
BIOSIMILAR / ADCC / CELL BASED ASSAY / DESIGN OF EXPERIMENTS / VALIDATION

BACKGROUND AND NOVELTY:
For the development of biosimilar monoclonal antibodies (mAb) or related substances containing the IgG Fc part it is mandatory to fully compare immunological properties between originator and biosimilar in a "comparability exercise". The most complex Fc associated function to mediate antibody dependent cellular cytotoxicity (ADCC) needs to be characterized using the active substance of the biosimilar and the comparator. The requirement to test for ADCC with high precision and accuracy is challenging. Design of cell lines to replace primary cells for effector or target cells is a solution to provide tools for standardized and extensive biosimilar testing.

EXPERIMENTAL APPROACH:
We developed a human transgenic NK-cell line with stable expression of Fc gamma-receptor IIIA (CD16) and stable functional characteristics. The NK-cell line shows advantageous properties for standardization of ADCC assays for monoclonal antibodies targeting CD20, HER-2/neu, membrane TNF alpha and EGFR using adherent and non-adherent target cells and various assay read-outs. We either selected target cells expressing the relevant antigen from established cell lines or generated these by stable genetic modification. Assays were developed by design of experiments to determine experimental factors of importance for assay suitability.

RESULTS AND DISCUSSION:
These tailored test systems provide suitable tools for validation and routine testing of various mAbs scalable to the analytical needs of biosimilar mAbs testing. We provide data showing system suitability and discuss precision and accuracy of our assay systems.

REF-D059

CASE STUDY: BIOSIMILAR ANTI TNFALPHA (ADALIMUMAB) ANALYSIS OF FC EFFCTOR FUNCTIONS

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KEY WORDS:
ADCC / BIOSIMILAR / ANTIBODY / CELL BASED / ASSAY

BACKGROUND AND NOVELTY:
For the development of biosimilar monoclonal antibodies or related substances containing the IgG Fc part it is mandatory to fully compare immunological properties between originator and biosimilar in a "comparability exercise". The important Fc associated functions to mediate antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) need to be characterized using both the active substance of the biosimilar and the comparator. For testing anti TNFalpha antibodies target cells with stable expression of the membrane TNFalpha is required. Further prerequisites are test systems facilitating analysis with high precision and accuracy.

EXPERIMENTAL APPROACH:
We generated a human transgenic NK-cell line with stable expression of Fc gamma-receptor IIIA (CD16) and stable functional characteristics to replace primary cells for effector cells in ADCC assays. Target cells for ADCC and CDC assays were genetically modified for stable expression of membrane TNFalpha (mTNFa) without the capability to release soluble TNFalpha. We analysed different batches of originators and a biosimilar candidate molecule for functional variability in both assays. The more complex ADCC assays were developed employing design of experiments.

RESULTS AND DISCUSSION:
We provide data showing assay suitability, precision and accuracy. Furthermore we discuss the variability of originator batches and biosimilar with respect to the assay capabilities.

DYNAMIC CULTURE OF HUMAN LIVER EQUIVALENTS INSIDE A MICRO-BIOREACTOR FOR LONG-TERM SUBSTANCE TESTING

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KEY WORDS:
MULTI-ORGAN-CHIP / TISSE ENGINEERING / ORGANOIDS / SUBSTANCE TESTING / LIVER

BACKGROUND AND NOVELTY:
The modeling of human liver tissue has gained much attention recently. Primary human cell culture systems are considered as a promising tool for evaluating the toxicology and pharmacology profiles of compounds metabolized by the liver. Current testing protocols using primary human cells in two-dimensional cell culture configurations are of limited biological relevance, as they do not adequately mimic the tree-dimensional environment of the liver. Therefore, we developed a characterized, bioreactor based, human in vitro tissue culture test system to overcome these obstacles. The miniaturized scale allows the cells to control their environment by secretion of autocrine and paracrine signals, through the very low medium-to-cell volume ratio. Steadier and more quantifiable extracellular conditions are provided by a microfluidic circulation, creating a specific physical microenvironment characterized by laminar flow close to physiological conditions.

EXPERIMENTAL APPROACH:
Human liver equivalents were generated by aggregating differentiated HepaRG cells with human hepatic stellate cells. These co-cultures were inserted into our dynamically perfused micro-bioreactor and cultured for up to 14 days at near physiologic fluid flow and volume to liquid ratios. The production of albumin, urea and lactate, as well as the consumption of glucose and the release of LDH into the culture medium was analysed daily. End-point analyses were performed by live-dead staining, immunofluorescence of marker proteins and RT-PCR of selected marker genes.

RESULTS AND DISCUSSION:
It could be shown, that the micro-bioreactor system is capable of supporting long-term co-cultures of human liver equivalents. Cell polarity was restored as shown by the expression of specific transporters, tight junctions and the formation of rudimentary bile canalicular like structures. Vitality of the cells was assessed by TUNEL/Ki 67 staining and was markedly increased compared to static controls.

SKIN AND HAIR-ON-A-CHIP: HAIR AND SKIN ASSEMBLY VERSUS NATIVE SKIN MAINTENANCE IN A CHIP-BASED PERFUSION SYSTEM

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KEY WORDS:
HAIR / SKIN EQUIVALENT / CHIP / PERFUSION / MICROFOLLICLE

BACKGROUND AND NOVELTY:
In recent decades, substantial progress to mimic structures and complex functions of human skin in the form of skin equivalents has been achieved. Different approaches to generate functional skin models were made possible by the use of improved bioreactor technologies and advanced tissue engineering. Although various forms of skin models are successfully being used in clinical applications, in basic research, current systems still lack essential physiological properties for toxicity testing and compound screening (such as for the REACH program) and are not suitable for high-throughput processes.

EXPERIMENTAL APPROACH:
In particular, further bioengineering is necessary for the implementation of adipose tissue, hair follicles and a functional vascular network into these models. In addition, miniaturization, nutrient and oxygen supply, and online monitoring systems have to be implemented in sophisticated culture systems. To become one step closer to the in vivo situation, we produced microfollicles as in vitro hair equivalents and integrated them into skin models. These microfollicles containing skin tissues were cultured under static and dynamically perfused conditions and were compared to ex vivo scalp and foreskin skin organ cultures.

RESULTS AND DISCUSSION:
Data on the successful integration of hair follicle equivalents into the skin models and their survival in long-term cultures will be presented. Furthermore, the extension of culture periods of integrated skin explants in the perfused chip-based bioreactor platform will be demonstrated. The current status of the development of a blood-perfused in vitro skin model and remaining hurdles will be discussed.

REF-D062

IDENTIFYING UROPATHOGENIC ESCHERICHIA COLI TRIGGERS OF IL-10 PRODUCTION IN URINARY TRACT INFECTION WITH UROTHELIAL HISTOTYPIC CO-CULTURES

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KEY WORDS:
URINARY TRACT / MICROBE / INFECTION / INTERLEUKIN-10 / HOST-PATHOGEN

BACKGROUND AND NOVELTY:

Urinary Tract Infection (UTI) affects 40% of women and results in ~10 billion dollars of health care costs in the USA alone. Uropathogenic Escherichia coli (UPEC) is the main UTI pathogen, typically causing cystitis following bladder colonization. Several aspects of host and pathogen mechanisms in UTI have been defined. In this study, we used a novel histotypic cell culture model of the human bladder to study these host-pathogen interactions as a closer mimic to in vivo conditions. We focused on the role of Interleukin-10 (IL-10) since recent work has described this in both murine UTI and adult UTI patients. The mechanisms underlying IL-10 synthesis and signalling triggered by UPEC are not yet understood.

EXPERIMENTAL APPROACH:

A novel histotypic cell culture model consisting of human urothelial, monocyte, B- and T-cells (90:10:1:1) was established and infected with UPEC at a multiplicity of infection of 10. Five-hour incubations were performed, and IL-10 levels were measured using ELISA. Parameters of the in vitro model were modified to determine the role of the different cell types and UPEC (live/heat-killed/ -irradiated) in the model that result in IL-10 synthesis. Experiments were performed to investigate the role of lipopolysaccharide (LPS) and flagella as IL-10 triggers by use of targeted mutants in UPEC genes encoding these factors (waaL, fliC).

RESULTS AND DISCUSSION:

Initial assays showed a typical two-fold increase in IL-10 protein following UPEC infection. A novel synergistic interaction between urothelial cells and monocytes was observed, whereby monocytes produce most IL-10 but the presence of urothelial cells amplifies monocyte IL-10 synthesis. Our findings showed that UPEC flagella and LPS contribute to the induction of IL-10, although other UPEC unknown factors also appear to contribute to this response. Overall, the data from these studies based on both murine and human models demonstrate the utility and value of histotypic models for investigating bacterial UTI pathogenesis.

IN VITRO SAFETY ASSESSMENT OF NANOSILVER WITH IMPROVED CELL CULTURE SYSTEMS

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KEY WORDS:
NANOSILVER / CACO-2 / M-CELLS / RISK ASSESSMENT

BACKGROUND AND NOVELTY:

In order to elucidate the complex interactions of food-related nanoparticles upon digestion in the gastrointestinal tract an improved in vitro cell culture system was set up. The model contain, beside the enterocytes, specialized microfold (M) cells, able to increase the absorption of micro- and nanoparticles (1, 2). In the current study the toxicity of nanosilver (AgNPs) was evaluated, reported to have the widest industrial applications (3).

EXPERIMENTAL APPROACH:

The co-culture model was received by co-culturing Caco-2 cells (clone 1, from Dr. M. Rescigno, University of Milano-Bicocca, IT) with RajiB cells (ATCC, Manassas, VA) in Transwell permeable supports (Corning Inc., NY) (1, 2). The cytotoxic effect of AgNPs < 20 nm (10-90µg/ml, Mercator GmbH, DE) was assessed by MTT assay. The barrier integrity of the cell monolayers of mono- and co-cultures under the influence of AgNPs was evaluated on 21 days fully differentiated cultures in bicameral inserts by measuring the transepithelial electrical resistance and the passage of Lucifer Yellow. The immunofluorescence staining of two tight junctions (TJs) proteins occludin and ZO-1 was realized by mouse anti-occludin/anti-ZO-1 as primary and Alexa Fluor 488 goat anti-mouse as secondary antibodies (Invitrogen). The images were collected by Zeiss LSM 710 confocal microscope.

RESULTS AND DISCUSSION:

AgNPs displayed a dose-dependent cytotoxic effect on Caco-2 cells starting from 30µg/ml, as well as led to a dose-dependent permeability increase of monolayers, likely connected with the changes in the TJs organization. AgNP-induced dashed and degraded distributions of both occludin and ZO-1, suggesting the opening of TJs. These effects were less obvious in co-cultures, a more accurate model to reflect in vivo conditions, suggesting that the presence of M-cells seemingly decreases the toxicity of AgNPs. Further improvement of the model by addition of e.g. mucus producing cells, dendritic cells will provide a tool to achieve even more realistic risk assessment of NPs in vitro.

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OTHER INFORMATION

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OTHER INFORMATION

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REF-D064

ADHESION AND COLONIZATION OF MESENCHYMAL STEM CELLS ON POLYLACTIDE OR PLCL FIBERS DEDICATED FOR TISSUE ENGINEERING

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KEY WORDS:
MESENCHYMAL STEM CELL / TISSUE ENGINEERING / SCAFFOLD

BACKGROUND AND NOVELTY:
Tissue engineering covers a broad range of applications dedicated to the repair or the replacement of part or whole tissue such as blood vessels, bones, cartilages, ligaments, etc. Pratically, a bio substitute, made with cells cultivated on scaffold, is needed. Mesenchymal stem cells (MSC) are generally the most suitable cells for such application since they are self-renewable with a great potential for differentiation and immuno suppression. However, materials used for bio functional scaffold synthesis have to meet several criteria as biocompatibility and biodegradability. Thus, the aim of the study was to screen several biopolymers differing in their composition and their capability to promote adhesion and growth of MSC.

EXPERIMENTAL APPROACH:
Porcine MSC were cultivated in a-MEM supplemented with 10 % serum and FGF2. For cell adhesion experiments, 6 (co)polyesters were synthesized and tested including commercial one (70 % L-lactic acid (LA), 30% caprolactone (CL) - PLCL comm.), MKG 58 PLCL (70 % D, L-LA, 30%-CL), MKG 64 PCL (polycaprolactone) PCL), MKG 70 PLCL (50 % L-LA, 50%-CL), MKG 71 PDLLA (poly(D,L-Lactide)), and MKG 74 PLLA ((poly(L-Lactide). Fibres of polymers were electrospun on 4 cm2 cover glasses, which were put onto 6 wells plate before to be seeded with MSC. Then, cell adhesion and colonization of polymer fibres were monitored by microscopy.

RESULTS AND DISCUSSION:
Whatever the polymer used, cells were able to adhere and to colonize fibres. Indeed, a cell multiplication factor ranging from 6.5 to 10 was measured after 200 hours of culture depending on the polymer composition. However, compared to the commercial PLCL, the total cell number was strongly increased with MKG 71 (50%), MKG 64 (43%) and MKG 58 (39%) whereas a moderate or no increase was observed with MKG 74 (16%) and MKG 70 (1%) respectively. Our results demonstrated that (co)polyesters composition strongly influences MSC behaviour and that polymers such as MKG 58, 64 and 71 could be favoured for further scaffold synthesis.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-D065

ONLINE METABOLISM MONITORING OF CHO CELLS CULTIVATED AS PERFUSION PROCESS IN SINGLE-USE BIOREACTOR (SUB) WITH CELLCORE MATRIX

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KEY WORDS:
PERFUSION / CHO-CELLS / ONLINE VIABLE CELL DENSITY / DIELECTRIC SPECTROSCOPY / SINGLE-USE BIOREACTOR

BACKGROUND AND NOVELTY:
Online monitoring of perfusion processes provides information of the growth of the cells, and can be used to control the perfusion parameters. Combining several analyzers yield information on the cell metabolism.

EXPERIMENTAL APPROACH:
Online analysers were applied on a 3 liter CellTank SUB with Chinese Hamster Ovary (CHO) cells harbored in 150 ml CellCore fiber matrix. All cells stayed inside the matrix, and therefore, it was not possible to sample cells for offline growth monitoring. A Fogale capacitance sensor was mounted with the tip inside the matrix, measuring the Viable Cell Density (VCD) online. Capacitance sensors only measure cells with intact cell membrane, and thus not the fiber matrix. A BlueSens gas-sensor measured the CO2 in offgas from the SUB. Glucose and lactate were monitored offline and used for control of perfusion rate. The specific production of IgG monoclonal antibody was determined daily. The temperature was lowered to lower the growth rate to lower media expense and accomodate the media handling.

RESULTS AND DISCUSSION:
VCD was showing logarithmic growth. CO2 concentration in offgas showed the initial pH-control and then CO2 formation had a precise correlation with VCD until lowering of temperature. The lower temperature gave a lower growth rate, but CO2 formation was lowered due to slower metabolism. Specific production of IgG followed CO2 formation. Dielectric spectroscopy indicated the cell proporties during cultivation and temperature change.
The perfusion culture produced 11 times higher amount of IgG antibody than a similar batch cultivation. However IgG was diluted compared to IgG in batch culture. Standard commercial media with fixed glucose concentration was used, and at high cell density of 80E6 cell/ml there was a high consumption of media.
Future studies will separate feed into media feed and concentrated glucose feed, which will give a higher IgG concentration at a lower cost, and the possibility to continuously run perfusion cultivation beyond cell density of 100E6 cells/ml.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Bent Svanholm has 10 years of fermentation experience from selling analyzers and sensors into the pharmaceutical industry.The expertise is being improved by upgrading an engineering bachelors degree to a Master in Biotechnology at DTU (Technical University of Denmark).
Acknowledgement to the Danish Agency for Science Technology and Innovation for funding the study performed at Bioneer A/S.
The SUBs were supplied by CerCell Biotech.

OTHER INFORMATION
Further online parameters has now been included for future studies: Online optical PCO2 and online HPLC.

DIFFERENTIATION INDUCTION OF K562 CELLS TO ERYTHROCYTES BY FLAVONOID APIGENIN

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KEY WORDS:
APIGENIN / ERYTHROCYTE / CELL DIFFERENTIATION / K562 / LEUKEMIA

BACKGROUND AND NOVELTY:

"Differentiation therapy" is one of the approaches in the treatment of cancer that is becoming popular recently, because of its potentially less toxic form of cancer therapy. Differentiation therapy usually involves the use of agents that modify the state and differentiation of cancer cells (Leszczyniecka et al., 2001; Jiang et al., 1994a). For cell differentiation studies involving leukemia, the K562 line has been used as a model cell line as it represents an early differentiation stage of the granulocyte lineage (Klein et al., 1976) is commonly used in studies on the induction of cell differentiation. Recently, we have reported that olive oil components can induce cell differentiation, and one of these is apigenin. Apigenin, a flavonoid abundant in parsley, olive fruits, and leaves, has been reported to have differentiation effects on leukemia cells.

We have previously reported that using proteomics analysis, treatment with apigenin stimulated the expression of proteins associated with the control of cell cycle, protein synthesis and folding, as well as for nuclear transport of signaling molecules. Also, the expression of GATA-1 gene was observed to increase in a time-dependent manner (Tsolmon et al., 2011).

EXPERIMENTAL APPROACH:

To further establish the cell differentiation potential of apigenin, we find out the level of expression of glycophorin (a protein present in the cell membrane of red blood cells) in apigenin-treated K562 cells using flow cytometry. In addition, since there are two types of hemoglobin, the fetal and the adult type, the type of hemoglobin present in the differentiated cells was also determined using real-time PCR.

RESULTS AND DISCUSSION:

Results show that the number of cells expressing glycophorin increased to 50% in apigenin-treated cells (compared to the control), 6 days following treatment with apigenin. To verify this result, the number of cells with hemoglobin was determined by staining the cells with benzidine and the results showed an increase in the number of cells that retained the stain in cells treated with apigenin for 10 days (compared to the control). These results in addition to the observed red color of the cell pellet clearly show that apigenin induced the K562 cells to become red blood cells; There are two types of hemoglobin, the fetal type (a2y2) and the adult type (a2ß2). To determine which type of hemoglobin was produced by the differentiated cells, real-time PCR was performed using specific primers for hemoglobin a, ß, and y. Results showed that treatment with 75 µM apigenin (treatment time: 2 days, 4 days, 6 days, or 8 days), increased the expression of hemoglobin a and ß 2 days after treatment with apigenin. However, treatment with apigenin for 8 days increased the expression of the hemoglobin a by 30-fold while the expression of hemoglobin y was increased 9-fold. The observed increase in the expre

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REF-D067

GENERIC STRATEGY FOR PHARMACOLOGICAL CAGING OF GROWTH FACTORS FOR TISSUE ENGINEERING

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KEY WORDS:
TISSUE ENGINEERING / GROWTH FACTORS / CAGING OF PROTEINS

BACKGROUND AND NOVELTY:
The possibility to trap small signaling molecules in cages and to subsequently uncage them in a controlled manner at the site of interest allows for the spatiotemporal manipulation of signaling processes. As many cellular processes rely on proteins rather than on small signaling molecules, the ability to cage proteins in a similar manner is highly desirable. Techniques potentially applicable to the caging of proteins have been reported; however, these are complicated and must be tailored for each specific protein of interest. A method enabling the caging of arbitrary proteins is thus much needed. We have developed a general procedure utilizing a cage to trap one or several proteins of choice. Moreover, to address the high demand to manipulate growth factor-controlled signaling pathways, we established a versatile platform consisting of the cage along with an optimized generic protocol for growth factor production.

EXPERIMENTAL APPROACH:
We devised a generic gene assembly and protein production platform using an analysis of variance (ANOVA) fractional factorial experimental design that allows for the customised production of arbitrary growth factors suitable for incorporation into the cage. The uncaging can be triggered on command, thus enabling temporal regulation of the process of interest.

RESULTS AND DISCUSSION:
The cage along with optimized growth factor production provides a versatile platform for exploring a variety of biological signaling processes through the precisely controlled uncaging of a bioactive compound. As an example illustrating the potential of this platform, caged growth factors were used for time-resolved stimulation of mesenchymal progenitor cell migration. The platform is believed to be valuable for fundamental and applied research ranging from elucidating signaling pathways to the targeted differentiation of cells in tissue engineering.

REF-D068

NOVEL PRIMARY-LIKE STROMA CELL LINES TO MODEL ASPECTS OF THE LEUKEMIA BONE MARROW (BM) MICROENVIRONMENT

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KEY WORDS:
LEUKEMIA / STROMA / CELL LINE

BACKGROUND AND NOVELTY:
For haematopoiesis an interaction of haematopoietic stem cells and stromal cells of the BM microenvironment is a prerequisite. In malignant disease such interactions can support the neoplastic cells by various mechanisms, e.g. blocking of cell death, proliferation induction, or suppression of anti-tumor immunity. The underlying mechanisms have just begun to be understood. To study features of leukemia stroma cells a novel in vitro model reflecting the in vivo situation was developed.

EXPERIMENTAL APPROACH:
We have established five stroma cell lines from BM samples of two patients suffering from acute myeloid leukemia (AML), two lymphoma patients, and a commercial sample from a normal donor (ND). This was accomplished with a technology that allows functional immortalization of the respective primary cells.

RESULTS AND DISCUSSION:
The cells have been in continous culture for >60 up to >100 passages, dependent on the different cell lines. In individual subclones of two lines a limited number of transgenes was found. Surface markers of the cells revealed a common MSC pattern. Differential expression was observed for immunoregulatory molecules, e.g. IL-10. Moreover, we studied immunomodulatory properties of cellular factors derived from the stroma cells on the maturation of dendritic cells (DCs). DC differentiation was monitored in the presence and absence of stroma cell fragments (SCF) obtained by sonification. Surface markers including CD40, CD80, CD83, CD86, CD274, and HLA-DR were investigated on monocytes, on day 3 after SCF addition, and on mature DCs. For maturation of DCs IL-4, GM-CSF, IL-1beta, and TNFalpha were added on day 7. A markedly decreased expression of the maturation marker and antigen-presenting molecule HLA-DR was found on DCs cultured in presence of AML stroma SCF compared to ND stroma SCF. This effect was not restored by addition of the maturation cytokines on day 7. In conclusion, these cell lines may be valuable tools for the in vitro study of features of a leukemic BM microenvironment.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
We thank Sana Mohamad, Stefanie Weber, and Sabine Schramm for expert technical assistance.

CHARACTERIZATION OF THE INFLUENCE OF CULTURE CONDITIONS ON EXTRACELLULAR MODIFICATIONS OF MONOCLONAL ANTIBODIES DURING FERMENTATION

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KEY WORDS:
MONOCLONAL ANTIBODY / FERMENTATION / MODIFICATION

BACKGROUND AND NOVELTY:
The production of protein-based medical agents, like monoclonal antibodies by biotechnological processes requires a comprehensive quality control. The pharmaceutical industry and national authorities support the complete characterization of therapeutic proteins to increase the quality and safety.
During numerous and different production steps like fermentation, purification and storage, various protein modifications on therapeutic products can occur. Modifications and their influences on functionality which occur during purification and storage are very well characterized. During the development of fermentation processes, good growth conditions for the cell culture are of primary importance to obtain maximal productivity [Müthing et al. (2003)]. Until now only few efforts have been made to investigate the development of extracellular antibody modifications and their sources during fermentation as the first phase of the productions process. Already known is the fact that pH-value and temperature can induce modifications on monoclonal antibodies [Usami et al. (1996)].

EXPERIMENTAL APPROACH:
Aim of this work is to increase the knowledge about the development of extracellular modifications of monoclonal antibodies during the fermentation process. Therefore, parameters of fermentation were identified which induce modifications during cell-free incubation under common fermentation conditions (shaker flask, and small scale bioreactor-system).

RESULTS AND DISCUSSION:
With this characterization a model was developed which predicts the quality of an antibody in dependence of different fermentation parameters. This model was confirmed for two monoclonal antibodies within numerous fermentations. As we aimed, with these results we gained an increased transparency of the fermentation process of monoclonal antibodies.

THE OPTIMIZATION OF A RAPID LOW-COST ALTERNATIVE FOR LARGE-SCALE MEDIUM STERILIZATION

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KEY WORDS:
MEDIA STERILIZATION / DEAD-END FILTERS / TFF / PUMPS / LARGE-SCALE

BACKGROUND AND NOVELTY:
One of the most important unit operations in upstream animal cell bioprocesses at scales over 100 L is the preparation and sterilization of the medium. This complex, sensitive, and expensive process requires a considerable investment in both material and time. Traditionally, large-scale medium sterilization is performed with costly single-use dead-end filters.

EXPERIMENTAL APPROACH:
In this study, an optimization of the cost and time for the sterilization of cell culture medium at volumes above 100 L was investigated. A range of different dead-end membrane filter materials including polyethersulfone (PES), polyvinylidene fluoride (PVDF), and mixed cellulose ester (ME) were tested using a positive displacement pump. Then, a range of different size glass microfiber (GF) pre-filters were tested in combination with and without the dead-end filters. In addition, tangential flow filtration (TFF) were examined was both a PES and ME membrane. Specific filtered medium volume, filter flux rate and filtrate turbidity were determined for each membrane type. Independently, pressure-volume diagrams were completed for both a positive displacement pump and a bearingless centrifugal pump to determined optimal pumping speeds and pressures.

RESULTS AND DISCUSSION:
This study showed that TFF coupled with the use of a bearingless centrifugal pump provides a rapid low-cost technology for the large-scale sterilization of cell culture medium.

REF-E001

DEVELOPING MULTI-GENE TARGETING METHODS FOR METABOLIC ENGINEERING IN CHO CELL FACTORIES

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KEY WORDS:
CHO CELL FACTORIES / TARGETED GENE INTEGRATION / GENE EXPRESSION

BACKGROUND AND NOVELTY:
The majority of protein therapeutics are produced from foreign genes that are integrated randomly into the genome of mammalian cells. Insertion of the foreign genes is traditionally achieved via virus-mediated or spontaneous integration of transfected DNA followed by selection of cells carrying the new genes. However, lack of control of gene insertion can give unwanted phenotypic heterogeneity due to the varying permissivity of integration sites for gene expression –also called position effect variation. Cell lines are therefore often unstable and show reduced fitness over time. Companies therefore screen thousands of clones for high producers that grow well and remain stable after many passages. It is therefore advantageous to target foreign genes into specific desirable sites in the CHO genome in a controlled manner to ensure high and stable expression.

EXPERIMENTAL APPROACH:
In this project, we are exploring the recently published CHO genome and proteome in order to develop sites that facilitate controlled insertion of foreign genes into chromosomal DNA using the DNA repair pathways of the cells. In order to speed up the insertion of many genes to engineer metabolism and glycosylation, DNA constructs and methods appropriate for large multi-gene pathway integration will be designed and prepared for CHO cell lines.

RESULTS AND DISCUSSION:
Preliminary experiments have identified suitable cloning methods for targeted gene insertion and synthesis of large gene constructs. These methods are currently being further developed and will be essential for future genome engineering and synthetic biology efforts in CHO.

REF-E002

GENERATION OF GENETIC ENGINEERED CHO CELL LINES TO SUPPORT THE PRODUCTION OF A DIFFICULT TO EXPRESS THERAPEUTIC PROTEIN

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KEY WORDS:
CELL LINE ENGINEERING / MICROARRAY / SHRNA / ZINC FINGER NUCLEASE / KNOCK OUT

BACKGROUND AND NOVELTY:
Chinese Hamster Ovary (CHO) cells are widely used for the large scale production of recombinant biopharmaceuticals. These cells have been extensively characterised and approved by regulatory authorities for production of biopharmaceuticals. During the last years more and more cell-line engineering strategies have been developed to enhance productivity and quality. CHO cell line engineering work has made incredible progress in optimizing products or titers by focusing on manipulating single genes and selecting clones with desirable traits. Here we present how cell line engineering enables the expression of an extremely difficult to express therapeutic protein.

EXPERIMENTAL APPROACH:
During the expression of a novel therapeutic protein significant reduced cell growth and productivity was detected. Using CHO specific microarrays the cause of reduced cell growth and productivity could be identified. Different cell line engineering approaches (knock down as well as knock out) were performed to circumvent cell growth inhibition and improve expression of the therapeutic protein.

RESULTS AND DISCUSSION:
Reduced cell growth inhibition is caused by a signal cascade resulting in down regulation of expression of mitochondria encoded genes. Different cell line engineering approaches were applied resulting in elimination of cell growth inhibition as well as down regulation of the expression of mitochondria encoded genes. Most striking is the improved productivity resulting in a more than seven fold titer increase using these genetic engineered cell lines. This example illustrates that cell line engineering is a powerful tool to solve definite project issues.

EVALUATING ZINC FINGER NUCLEASE (ZFN) EFFICIENCY ACROSS SPECIES AND GENOMIC LOCI IN CHINESE HAMSTER OVARY CELLS TO IMPROVE TARGETED INTEGRATION RATES

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KEY WORDS:
CHO / TARGETED INTEGRATION / ZINC FINGER NUCLEASE / CHROMOSOMAL CONTEXT

BACKGROUND AND NOVELTY:

Zinc Finger Nucleases (ZFN) have provided researchers with a tool for site–specific modification of endogenous genomic sequences. ZFN's are used to introduce double strand DNA breaks which can be exploited to insert exogenous elements into cell lines. ZFN-mediated integration rates are dependent on chromosomal structure, ZFN cutting efficiency and host cell line. Site-specific integration of r-protein expression constructs into genomic “hot spots” may lead to increased cell line stability, decreased system heterogeneity and shorter cell line development timelines. Our goal is to increase ZFN-mediated integration rates in CHO through the integration of an engineered landing pad. This is the first reported study comparing the effects of chromosomal location on exogenous ZFN activity as well as evaluating ZFN efficiencies across different species.

EXPERIMENTAL APPROACH:

We created the landing pad using the human AAVS1 locus because the recognition sequence doesn’t exist in CHO and the human AAVS1 ZFN’s are highly efficient (typical efficiencies of >20% in human cells lines). The landing pad was integrated into three different loci in the CHO genome. Each of the CHO loci targeted has varied endogenous ZFN activity. We selected Rosa26 (low ZFN efficiency, 10%) the Neu3 locus (mid-level ZFN efficiency, 5-10%) and a third proprietary DNA sequence designated site 3 (high-level ZFN efficiency, >10%).

RESULTS AND DISCUSSION:

In this study we established rates of ZFN-mediated targeted integration (TI) into the Chinese Hamster Ovary (CHO) genome of 1-3 % when using ZFN’s with average cutting efficiencies of 15%. CHO clones carrying the exogenous human AAVS1 ZFN recognition sequences were isolated, and single or biallelic integrations were confirmed by junction PCR and sequencing. We have confirmed AAVS1 ZFN activity directly across these three engineered CHO loci as well as at the native loci in human cells. Exogenous ZFN cutting efficiencies vary based on CHO genomic context and chromosomal location.

A QUICK AND ROBUST METHOD FOR SINGLE-CELL CLONE GENERATION IN THE HUMAN CAP CELL LINE FOR PROTEIN PRODUCTION

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KEY WORDS:
SINGLE-CELL CLONING / HUMAN CELL LINE / SUSPENSION / SERUM-FREE

BACKGROUND AND NOVELTY:

For the production of biotherapeutics, suspension cells have become a highly valuable tool. They offer the necessary scalability for large scale production and are relatively easy to handle in industry-scale upstream processes.
However, generating single-cell derived clones from suspension cells can be difficult and time consuming. For several cell lines, single-cell clone generation is done at the adherent stage and adaptation to suspension culture is subsequently performed on selected clones. Other cells are cloned in suspension but require the presence of feeder cells and/or conditioned media. For the human cell line CAP, which has been optimized for the production of biotherapeutic proteins, we have developed a rapid and reliable serum-free single cell cloning protocol which circumvents these hurdles.

EXPERIMENTAL APPROACH:

Several single cell cloning protocols were tested, including limiting dilution before and after antibiotic selection, mixtures of different media at different ratios, the addition of several supplements during the process and different cell passaging strategies.

RESULTS AND DISCUSSION:

After several rounds of optimization, a protocol was developed which considerably shortened the timelines from CAP cell transfection to an isolated, characterized single-cell clone.. In addition, with the new protocol cloning efficiency was increased to from 10-20% to 40-50%.
The protocol works with cells growing in suspension and has no requirement for either conditioned media, serum or feeder cells. It has significantly sped-up and simplified cell line generation from CAP cells and can possibly be transferred to other cell types as well.

REF-E005

NOVEL WORKFLOW FOR PARALLEL HIGH-THROUGHPUT SCREENING OF POLYMER VECTORS FOR NON-VIRAL GENE DELIVERY

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KEY WORDS:
HIGH TROUGHPUT / NON-VIRAL GENE DELIVERY / POLYMER / POLYETHYLENIMINE / COMBINATORIAL WORKFLOW

BACKGROUND AND NOVELTY:
Within the last years, high-throughput (HT) becomes a keyword in polymer research for gene delivery. Hence, polymer properties, e.g., molar mass, functional groups, architecture, or the combination of different monomers in statistic or block copolymers can be altered, yielding polymer libraries which allow the examination of structure property relationships.[1-3] But unfortunately, the subsequent biological evaluation of the prepared polymers is still time consuming and limited regarding a combinatorial HT workflow.[4]

EXPERIMENTAL APPROACH:
We present a novel HT workflow for the investigation of cationic polymers for gene delivery. Therefore, various linear and branched polyethylene imines (PEI) were used as representative vectors and investigated via HT assays in a 96-well plate format, ranging from the polyplex preparation up to the examination of the transfection process. The HT workflow starts with the automated polyplex preparation via pipetting robots and continues with a parallel and HT analysis of the size, binding affinity, stability, transfection and toxicity of prepared polyplexes.

RESULTS AND DISCUSSION:
The automatic approach of the biological studies permits the screening of manifold parameters of different polymers in terms of their transfection properties. Moreover, the potential of the HT analysis will be shown by using further polymer classes, such as methacrylates. The presented workflow represents a great facility to gain deeper insights into cationic polymers regarding their physiochemical properties and biological parameters, as transfection efficiency and cytotoxicity.

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REF-E006

EFFICIENT TRANSFECTION OF SUSPENSION CELLS BY STIMULI-RESPONSIVE TRIBLOCK TERPOLYMER MICELLES

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KEY WORDS:
TRANSFECTION / SUSPENSION CELLS / MICELLE / NON-VIRAL GENE DELIVERY / ENDOSOMAL BURST

BACKGROUND AND NOVELTY:
The controlled delivery of genetic material into eukaryotic cells has been the focus of interdisciplinary scientific activities during the last two decades. Beside evolutionary qualified and very efficient viral transfection, non-viral delivery is of high interest, reflected in the large number of non-viral transfection agents being proposed. Thereby, the cationic polymer poly(ethylene imine) (PEI) represents the “gold standard” for in vitro applications. Several studies show that the polymer architecture and the overall molar mass have major impact on the transfection efficiency.[1-3] Nevertheless, it is still challenging to design systems comprising high TE and low cytotoxicity for non adherent cells used in biotechnology.

EXPERIMENTAL APPROACH:
We demonstrate the use of multicompartment micelles formed via self-assembly of a stimuli-responsive triblock terpolymer, poly (butadiene-block-poly (methacrylic acid)-block-poly (2-dimethy-laminoethyl meth¬acrylate), as promising transfection agents. The hydrophobic PB forms the micellar core which, at low pH, is surrounded by a PMAA shell and a PDMAEMA corona. Such micelles are dynamic and show a strong pH dependence concerning shape, size, and surface charge.[4]

RESULTS AND DISCUSSION:
The low cytotoxicity and high TE for both adherent and suspension cells will be demonstrated. In particular in the latter case, remarkable improvements compared to PEI and linear PDMAEMA will be shown. Detailed investigations of the underlying mechanism revealed several advantages for the new system: the dense core of the BMAAD micelles leads to higher sedimentation rates and an increased cellular uptake. Furthermore, the interplay of two oppositely charged weak polyelectrolytes (PMAA and PDMAEMA) within shell and corona is responsible for a higher viability and an improved pDNA release. In combination, these effects render BMAAD a powerful advanced carrier for transient pDNA transfection studies of suspension cells.

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POLYCATIONIC NANO-PARTICULATE AGENTS FOR THE EFFECTIVE DELIVERY OF PDNA AND SIRNA TO DIFFERENTIATED CELLS AND PRIMARY HUMAN CELLS

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KEY WORDS:
NON-VIRAL GENE DELIVERY / SIRNA / PRIMARY CELLS

BACKGROUND AND NOVELTY:

The delivery of polynucleotides such as plasmid DNA (pDNA) and siRNA to non-dividing and primary cells by non-viral vectors presents a considerable challenge. In recent years, we have seen a number of studies, which link size and structure of non-viral polycationic transfection agents to their performance. Increasing size of the polycation often correlates with improved transfection efficiency, but also with an increase in cytotoxicity. Concomitantly, evidence is building up that non-linear polymer structures are more efficient transfection agents than linear polymers of the same size (e.g., Synatschke et al., 2011). By using modern polymer chemistry, a precise tailoring of functional groups and topology of the polymers are available. Here, we present novel polycationic nanoparticles based on multi poly(2-(dimethylamino)ethyl methacrylate (PDMAEMA) - based arms emanating from a common center.

EXPERIMENTAL APPROACH:

These nanoparticles were used to deliver nucleic acids in non-dividing cells and also in human primary cells. The polyplexes built at various NP ratio were transfected under serum-free conditions and the expression of the transgene or the knock-down of the targeted gene were analyzed by flow cytometry.

RESULTS AND DISCUSSION:

The nanoparticles show, to our knowledge, a unique capability for the transfection of primary and non-dividing cells by adequate biocompatibility (Schallon et al., 2012). The novel agents can furthermore efficiently deliver siRNA into primary human cells, in particular in T lymphocytes, and into recombinant cell lines leading to the specific knock-down of the targeted gene. Therefore, these novel agents are expected to be promising carriers for non-viral gene transfer.

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INVESTIGATION OF NEW CARRIERS FOR NON-VIRAL GENE DELIVERY

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KEY WORDS:
NON-VIRAL GENE DELIVERY / STAR-SHAPED NANOPARTICLES

BACKGROUND AND NOVELTY:

The basis of genetic modification of mammalian cells ("transfection") is the introduction of nucleic acids into the cells. This involves an efficient uptake of the pDNA as well as its delivery into the nucleus. For the non-viral gene delivery, cationic polymers can be used as delivery system although transfection efficiency and biocompatibility highly depend on the polymer chemistry and structure. In recent years, evidence is building up that non-linear polymer structures are more efficient transfection agents than linear polymers of the same size (e.g., Synatschke et al., 2011). In particular, we showed that PDMAEMA-based star-shaped nanoparticles synthesized from an inorganic (PDMAEMA230/20) or a polybutadiene core (B290D245) display high potentiality for transfection of primary and differentiated cells as well as a more efficient pDNA delivery into standard cell lines compared to PEI. Thus, this work established that the design principle of many arms emanating from a common center results in efficient polynucleotide delivery vehicles independent of the core material and, therefore, offers advanced possibilities for the development improved gene vectors, in particular, for primary cells. [Schallon et al., 2012]. But so far, the mechanisms responsible for the outstanding transfection capability of the PDMAEMA-based star-shaped nanoparticles are still unknown.

EXPERIMENTAL APPROACH:

Preliminary characterized (zeta potential and DLS) polyplexes were transfected under serum-free conditions in standard cell lines.

RESULTS AND DISCUSSION:

The interaction of positively-charged polyplexes with the cell membrane is usually postulated as the basis of an effective uptake in the cells. Here, we present a systematic analysis of the polyplexes physico-chemical properties (e.g., net charge, size distribution,…) in correlation with the transfection outcome. Furthermore, analysis of the influence of the culture mode (adherent / suspension) as well as the presence of surface proteins at the time of transfection is discussed.

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REF-E010

HIGH PERFORMANCE CHO CELL LINE DEVELOPMENT PLATFORM FOR ENHANCED PRODUCTION OF RECOMBINANT PROTEINS INCLUDING DIFFICULT TO EXPRESS PROTEINS

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KEY WORDS:
RECOMBINANT PROTEINS / DIFFICULT-TO-EXPRESS PROTEINS / CELL LINE DEVELOPMENT

BACKGROUND AND NOVELTY:
Traditionally, the drug discovery process includes a variety of successive systems for candidate screening until the identification of a lead compound. Production systems such as bacteria, yeast or cell-free systems remain widely used for convenience reasons. Transient methods in mammalian cells are also routinely used for difficult-to-express products in simpler systems and provide research suitable yields. However, this approach relying on system switches is time consuming, cost-effective and may impact the product quality and efficacy. The development of novel comprehensive mammalian expression platforms thus represents a growing need for advancing biopharmaceuticals towards human clinical trials. Our goal is to offer a novel discovery solution that allows the use of stably transfected Chinese hamster ovary (CHO) cell lines as a tool to rapidly identify lead therapeutic candidates.

EXPERIMENTAL APPROACH:
We have built a fully integrated platform covering each step from genetics to process optimization and based on the combination of chromatin remodeling elements (Selexis Genetic Elements) and high transfection efficiency with a potent host Chinese Hamster Ovary (CHO) cell line. This approach allows the identification of relevant protein candidate variants and high expressing clones at the same time, thus addressing activity and productivity simultaneously for faster and improved protein library screening campaigns. Our approach relies on a fully integrated platform covering each step from genetics to process optimization.

RESULTS AND DISCUSSION:
Altogether, these approaches customized for each project better support clonal cell line performance in terms of specific productivity, cell viability and product quality. This novel platform offers considerable time and labor savings as it enables the development of the production clonal cell line directly from the pool of transfectants in optimized culture conditions. Based on this approach, combinatorial human antibody libraries are generated in CHO cells.

REF-E011

NUTRITION AND EPIGENETICS?

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KEY WORDS:
DNA METHYLATION / INTERLEUKIN-8 / CACO-2 / PYROSEQUENCING / PLANT INGREDIENTS

BACKGROUND AND NOVELTY:
Evidence is increasing that nutrition affects human healthy. Some foods (vegetables, green tea) are correlated with positive effects on several diseases. Influence on cytokine levels in cell culture studies are reported for some plant ingredients. The pseudocereal plant Amaranthus caudatus, originally cultivated in South America, contains large amounts of proteins, polyunsaturated fatty acids, minerals and iron, but no gluten. Furthermore Amaranth contains squalen and tocotrienols that affect cholesterol synthesis. Diets with Amaranth also have been assessed in clinical studies for patients with celiac disease and immunodeficiencies. To examine the effects of Amaranth ingredients we focused on interleukin-8 (IL-8) protein expression and gen alteration in gut cells in vitro.

EXPERIMENTAL APPROACH:
Cells of the colon carcinoma cell line Caco-2 were differentiated and incubated with control substances like folic acid and butyrate. Samples were taken from Amaranthus extracts and incubated for 24 or 48 hours. Culture supernatants were collected and IL-8 expression was determined with an ELISA. DNA was extracted from the cells followed by a bisulfite conversion and pyrosequencing.

RESULTS AND DISCUSSION:
Butyrate, folic acid and LPS (Lipopolysaccaride) increased IL-8 contents in supernatants whereas a combination of LPS and Amaranth extracts reduced soluble IL-8 protein. We examined the methylation grade of seven CpG sites (DNA methylation occurs on specific sites, so called CpG sites, where a cytosine is followed by a guanine in the DNA sequence) in the promoter region and two CpG sites upstream specific for the IL-8 gen. Due to a high methylation grade of the controls, specific effects of Amaranth samples could not be observed. Methylation in the CpG sites can alter transcription of a gen and protein expression but has to be studied in cells with low basic methylation.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-E012

NOVEL SPLICE VARIANTS OF CHO CELLULAR AMYLOID PRECURSOR PROTEIN MRNA AND METHOD FOR SPECIFIC DETECTION OF THESE VARIANTS IN A BIOLOGICAL SAMPLE

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KEY WORDS:
AMYLOID PRECURSOR PROTEIN / CHO CELLS / SPLICE VARIANTS / MRNA

BACKGROUND AND NOVELTY:
Amyloid precursor protein (APP) is an integral membrane protein expressed in different cells/tissues in many organisms. Proteolysis of APP generates beta amyloid (A) peptide which is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients. It has been previously reported that Chinese hamster overy (CHO) cells express full length APP, but no detailed information about CHO APP gene structure/expression in K1-cellline yet available. In addition, no alternative splicing isoforms of APP have yet been observed in CHO cells.

EXPERIMENTAL APPROACH:
Novel splicing testing methods based on the amplification of cell sample derived mRNA have been developed. These methods allow for improved detection limits of spliced RNA variants in biological samples.

RESULTS AND DISCUSSION:
Described is the identification of APP splice variants resulted from alternative splicing of the APP mRNA in CHO-K1 cells. At least four (4) different APP transcripts have been identified, and these mRNA variants occur through skipping one or more full exons within the APP gene. The presentation also describes certain general methods for the detection of these RNA variants in a biological sample.

REF-E013

A COMPARATIVE ANALYSIS OF CHINESE HAMSTER VERSUS CHO CELL LINE TRANSCRIPTOME USING RNA-SEQ

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KEY WORDS:
RNA-SEQ / CHO / HAMSTER TISSUE / TRANSCRIPTOMICS / BIOINFORMATICS

BACKGROUND AND NOVELTY:
The Chinese hamster ovary (CHO) cell-line is the predominant mammalian industrial cell line being used to produce recombinant therapeutic proteins today. Previous karyotyping studies and bacterial artificial clone (BAC) library sequencing have discovered that CHO cell lines have fewer chromosomes than Chinese Hamster cells and that CHO cell lines undergo extensive chromosomal rearrangements during cell line creation due to gene amplifications. With the recent sequencing of the ancestral CHO-K1 cell line genome it has now become possible to catalog and compare differences between various CHO cell lines and primary Chinese Hamster tissue. In order to better understand the gene regulatory characteristics of CHO cells we have undertaken transcriptome profiling of several samples.

EXPERIMENTAL APPROACH:
We have obtained between 2.32 – 9 Gbp of RNA-seq transcriptome sequences for each of six Chinese Hamster tissue samples (brain, kidney, liver, lung, ovary and spleen) and two CHO cell lines (CHO-K1 and an anti-Her2 producing recombinant cell line called CHO-SH87). We used the recently released CHO-K1 draft genome as a reference for this analysis and then performed a reference-free assembly of the transcriptome samples using the Trinity software package. A differential gene analysis was carried out between the eight transcriptome samples using the Cufflinks RNA-seq processing software.

RESULTS AND DISCUSSION:
By performing a reference free transcriptome assembly we were able to add to and improve the current CHO-K1 reference transcriptome collection. Our differential analysis revealed diverse changes in gene expression within the group of tissue samples, and also several differences between tissue and cell line gene expression. In addition to discovering phenotypic differences between the cell samples based on gene expression alone we also used this data to infer metabolic differences between the samples, by combining gene expression data with a CHO metabolic network model.

REF-E014

INTEGRATED MIRNA, MRNA AND PROTEIN EXPRESSION ANALYSIS REVEALS THE ROLE OF POST-TRANSCRIPTIONAL REGULATION IN CONTROLLING CHO CELL GROWTH RATE

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KEY WORDS:
CHINESE HAMSTER OVARY / GROWTH RATE / MICRORNA / MRNA / PROTEOMICS

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are a class of small RNA molecules involved in the post-transcriptional control of protein synthesis. Recent studies have linked miRNA expression to several industrially important traits in Chinese hamster ovary (CHO) cell culture. This study represents the first time that simultaneous analysis of miRNA, mRNA and protein abundance levels has been carried out to understand the role of miRNA in the regulation of CHO cell growth.

EXPERIMENTAL APPROACH:
qPCR, microarray and quantitative LC-MS/MS analysis were utilised for expression profiling of miRNA, mRNA and protein levels for a set of sister MAb-producing clones spanning a range of growth rates. The proteomic and mRNA data were initially analysed in isolation to identify enriched biological processes. To investigate the influence of miRNA on these processes we combined the proteomic and transcriptomic data into two groups. The first set contained proteins where evidence of translational repression was observed. The second group was a mixture of proteins and mRNAs where translational repression was less clear. TargetScan was utilised to predict targets within these two groups for anti-correlated differentially expressed (DE) miRNAs.

RESULTS AND DISCUSSION:
Gene ontology analysis of genes (n=432) and proteins (n=285) found to be DE identified biological processes driving proliferation including mRNA processing and translation. Following the integration of protein and mRNA data a number of proteins central to these processes including several hnRNPs and components of the ribosome were found to be post-transcriptionally regulated. Comparison of mRNA and protein expression with respect to the 51 DE miRNAs (35 miRNAs up and 16 miRNAs down) allowed us to identify potential miRNA targets and highlight translational repression targets which could not have been identified using a single dataset. Moreover, the use of multiple profiling datasets could allow the identification of non-seed miRNA targets and reduce false positive/negative rates.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-E015

ASSESSMENT OF CHROMOSOMAL LENGTH VARIATION IN CHO CELLS

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KEY WORDS:
CHROMOSOME / CHO CELLS / METAPHASE / KARYOTYPE / DIGITAL IMAGING

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO)-derived cells are widely used for the production of recombinant proteins. Currently, the pharmaceutical industry relies on stable cell clones for therapeutic protein production. Most of the currently used methods to analyze clonality and stability in recombinant cell lines only take into consideration the cellular phenotype. However, few rapid methods are available to study clonality and genomic stability in recombinant cell populations.

EXPERIMENTAL APPROACH:
Here we present a relatively simple, cost-effective method based on digital image processing to analyze chromosome lengths in metaphases of cells. The method was applied to CHO parental cells and derived recombinant cell lines. Chromosome spreads were prepared from growth-arrested cells and visualized with a confocal microscope after fluorescence staining. An ImageJ plugin was then used to size-sort the chromosomes.

RESULTS AND DISCUSSION:
We studied 9 different CHO derived cell lines, both parental and recombinant sublines (20 metaphase spreads for each cell line). The average chromosome number in each clonal line was not homogenous. Also, each cell line appeared to have a unique pattern of chromosome length. We believe that the analysis of chromosome length patterns of CHO lines can be useful in the characterization of a given cell population and possibly even in the identification of the parental cell line from which a subline is derived from a specific parental cell.

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SHORTENED TIMELINE FOR CELL LINE DEVELOPMENT - ADAPTION OF RECOMBINASE-MEDIATED CASSETTE EXCHANGE (RMCE) TO SUSPENSION CHO CELLS

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KEY WORDS:
RMCE / CELL LINE DEVELOPMENT / FLP-RECOMBINASE / MAB

BACKGROUND AND NOVELTY:
The first step in manufacturing recombinant glycosylated therapeutic proteins is the search for a high and stable expressing cell clone. In contrast to traditional approaches for cell line development Fraunhofer ITEM developed an alternative strategy called recombinase-mediated cassette exchange (RMCE). Traditional concepts for the development of cell lines are based on random integration of the expression vector containing the gene-of-interest (GOI) which makes the screening for an appropriate cell clone very time and labor intensive. The application of the RMCE-strategy based on the site-directed integration of a single copy of the gene-of-interest into a transcriptional, exchangeable and known genomic locus dramatically shortens the timeline for the cell line development.

EXPERIMENTAL APPROACH:
In order to mark the genomic locus, CHO-cells were initially transfected with either a lentiviral or plasmid based Tagging vector containing a selection and a fluorescent marker flanked by two heterospecific recombination target sites. The transfected cell pool was subsequently screened for highly expressing, stable cell clones with an exchangeable locus. The following exchange of the Tagging versus the Targeting cassette, carrying the gene-of-interest (GOI), was catalysed by Flp-Recombinase. Enrichment of the GOI-producing cell population was made without drug selection. Targeted clones were further analyzed concerning their productivity, growth, long-time stability.

RESULTS AND DISCUSSION:
Both lentiviral and plasmid tagged CHO suspension cells were targetable and showed high GOI expression after two rounds of selection without antibiotics. Targeted clones were able to be cultivated in suspension again which allows a potential scale-up. PCR analysis further verified correct exchange and integration of the Targeting vector. Additional experiments are in progress to examine whether a certain genomic locus is suitable to express only certain protein classes.

OPTIMIZATION OF A LENTIVIRAL VECTOR FOR TRANSIENT AND STABLE PROTEIN OVEREXPRESSION IN CHO AND HEK 293 CELL LINES

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KEY WORDS:
PROTEIN EXPRESSION / LENTIVIRAL VECTOR / ANIMAL CELLS / LEADER INTRON / OPTIMIZATION

BACKGROUND AND NOVELTY:
Recombinant protein overexpression in animal cells constitutes a real challenge in the biomolecule production for therapeutic purposes. Following the intron functionality discovery as gene expression enhancers, various expression vectors that include them in their sequences have been developed. Generally, these are located in the 5' untranslated region (5'UTR), in which case they are called leader introns. In the present work, we have studied various systems leading promoters (CMV or EF1a) and 5' UTRs sequences (HsEF1-a, human Elongation Factor 1-a; CgEF1-a, C. griseus Elongation Factor 1-a; CMV, cytomegalovirus immediate-early 1 gene; CI, Chimeric Intron from the 5'-donor splice site from human ß-globin intron 1 plus the 3'-acceptor splice site from the intron of an immunoglobin gene heavy chain variable region) in different combinations, fused to either a gene coding for green fluorescence protein (GFP) or recombinant human Factor VIII (rhFVIII).

EXPERIMENTAL APPROACH:
Analyzed sequences were amplified by PCR using appropriate primers and templates. Then they were cloned in lentiviral vectors that previously contained either GFP or rhFVIII genes. GFP levels were evaluated by flow cytometry and rhFVIII productivities by sandwich ELISA, after transfection or transduction assays.

RESULTS AND DISCUSSION:
When evaluating GFP and rhFVIII levels in transient and stable expression conditions in CHO-K1 and HEK293 cell lines, we were able to identify specific promoter/5'UTR combinations with higher expression levels compare to commercial vectors. Expression levels depended on the evaluated expression platform and cell line, reaching increments between 2.5 and 4 times and 1.5 to 4 times in transient and stable conditions, respectively.
In summary, in this study we were able to develop new lentiviral vectors for protein overexpression that achieved superior expression levels with respect to widely used vectors and constitute promising candidates as gene expression systems in animal cells in transient and stable conditions.

REF-E018

ROOT CAUSE ANALYSIS OF THE LOSS OF MONOCLONAL ANTIBODY PRODUCTIVITY IN CHO CLONES OVER TIME

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KEY WORDS:
CELL LINE STABILITY / CHINESE HAMSTER OVARY / MONOCLONAL ANTIBODY

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO) cells are the most widely used mammalian cell line for production of therapeutic proteins exhibiting high productivities in the gram per liter range. Responsible cellular mechanisms of how cells manage to produce, fold, modify and export these large protein quantities properly and especially causes for the loss of production over time still remain largely unknown. Recently, the CHO genome was sequenced, which allows the use of molecular biologic approaches to identify potential genes and pathways involved.

EXPERIMENTAL APPROACH:
A monoclonal antibody (mAb) project was chosen which harbored a tendency for instable clonal cell populations. 45 clones expressing this mAb were cultivated over 12 weeks and antibody expression was assessed throughout this study. Clones were defined unstable if antibody titers decreased by more than 30%. Genomic DNA and mRNA levels of heavy chain (HC) and light chain (LC), karyotypes and plasmid integration sites of selected stable and unstable clones were analyzed.

RESULTS AND DISCUSSION:
Out of 45 CHO clones 24% proved stable and 76% unstable. Reduced titers during 12 weeks cultivation correlate with reduced expression levels of HC mRNA which leads to the assumption that limited HC mRNA levels cause reduced antibody production. Unstable clones loose HC copies on genomic DNA level over time whereas the copy number of the LC appears constant. FISH data reveal the integration locus of transfected plasmids and that the loss of HC copies is not due to the loss of chromosomes. Hybridization data confirm that only parts of the integrated plasmids are lost. We hypothesize that the deletion of HC genes is due to homologous recombination. Further molecular biologic methods to analyze these clones are planned and analysis thereof will shed light on the impact of regulated genes on cellular pathways and mechanisms which influence the expression of therapeutic proteins and cell line stability.

REF-E019

MESSENGER RNA AND MICRORNA EXPRESSION PROFILING OF HIGH AND LOW PRODUCING RECOMBINANT CHINESE HAMSTER OVARY CELL LINES

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KEY WORDS:
CHO CELLS / TRANSCRIPTOMICS / MICROARRAY / MRNA / MIRNA

BACKGROUND AND NOVELTY:
Recombinant Chinese hamster ovary (CHO) cells are the most frequently applied mammalian expression system for the production of therapeutic proteins. Although CHO cells have been used for more then 25 years, the cellular processes that control and limit recombinant protein production and secretion are poorly understood. Today, microarray technology is a common tool to analyze transcriptional activity. Samples for transcriptome analyses are generally taken in the exponential growth phase. However, the cellular transcriptome is very dynamic during batch cultivation where the conditions change continuously. For this reason steady state cultivation was applied in this study. This enables the generation of high quality mRNA and miRNA expression data which are ideally suited for a comparative analysis of distinct cell lines.

EXPERIMENTAL APPROACH:
High and low producing recombinant CHO cell lines for two model proteins (human serum albumin and the single chain Fv-Fc fusion antibody 3D6scFv-Fc) were developed by selecting stable clones at different stages of posttransfectional gene amplification. The cell lines were then cultivated in a chemostat process. This allows the establishment of steady-state conditions and the specific growth rate can be controlled by the dilution rate. The differences in mRNA expression patterns of high, low and non producing recombinant CHO cell lines were studied in a microarray experiment. For this purpose a CHO specific microarray was designed. Equally, miRNA expression was analyzed using a miRNA microarray.

RESULTS AND DISCUSSION:
Comparing the expression patterns of the high, low and non producers revealed several differentially expressed genes and miRNAs that are potentially involved in the cellular control of protein production and secretion. In addition, both datasets were analyzed in order to find correlations between mRNA and miRNA expression. Functional pathway analysis was conducted and the significance of enriched pathways for recombinant protein expression will be discussed.

DYNAMIC PROFILING OF AMINO ACID TRANSPORT AND METABOLISM IN CHINESE HAMSTER OVARY CELL CULTURE

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KEY WORDS:
AMINO ACID TRANSPORTERS / TRANSCRIPTOMICS / EXTRACELLULAR AND INTRACELLULAR AMINO ACID CONCENTRATIONS / FEEDING STRATEGY / BIOPROCESSING

BACKGROUND AND NOVELTY:
Following the recent publication of the Chinese hamster ovary (CHO)-K1 genome, a key piece of the puzzle will be unravelling its functionality through -omics analyses, which will also provide basis for comparing various CHO-derived hosts. We have focussed our study on elucidating amino acid (a.a.) metabolism following a combined transcriptomic and metabolomic approach. The cellular regulation of a.a. is of paramount importance and not yet fully understood, especially for the glutamine synthetase (GS) CHO cell lines.

EXPERIMENTAL APPROACH:
On the transcriptomic side, a global study of 40 a.a. transporters (out of a total of 46 genes associated with a.a. transport) in a blank and two recombinant protein-producing GS-CHO cell lines (a medium and a high producer) has been conducted using real time quantitative reverse transcription polymerase chain reaction on samples from different phases of batch cell culture. The metabolomics approach focussed on identifying the extracellular and intracellular a.a. profiles using high performance liquid chromatography.

RESULTS AND DISCUSSION:
The results reveal that ~30% of transporters are lowly expressed (fractional copies per cell), 9% are below levels of detection, whereas 40% are significantly differentially expressed either during batch cell culture, or between cell lines, or both. The remaining transporters appear to remain stable. Our data point out several genes upregulated towards stationary phase of cell culture in all cell lines, suggesting the need of the cells to either import or export the relevant substrates. A small number of transporters are present in significantly higher levels in the recombinant cell lines and can be targeted for genetic engineering approaches. The a.a. transporters gene expression findings correlate well with the extracellular and intracellular concentration profiles of their respective substrates. The latter pinpoint essential amino acids that are exhausted by the stationary phase and enable the mapping of transport and metabolism.

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BOYE	Jean-louis	Merck Serono	Coinsins	SWITZERLAND
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BRANDL	Melanie	Merck Kgaa	Darmstadt	GERMANY
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KRÜGER	Olaf	Biotest Ag	Dreieich	GERMANY
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KUCHIBHATLA	Jagdish	Kerry Inc.	Beloit	UNITED STATES OF AMERICA
KUENG	Stephanie	Roche	Basel	SWITZERLAND
KÜHNER	Markus	Adolf Kühner Ag	Birsfelden (Basel)	SWITZERLAND
KÜHNER	Laura	Adolf Kühner Ag	Birsfelden (Basel)	SWITZERLAND
KUNERT	Renate	University Of Natural Resources And Life Sciences	Vienna	AUSTRIA
KURODA	Shuncihi	Nagoya University	Nagoya	JAPAN
KUSANO	Setsuko	University Of Tsukuba	Tsukuba City	JAPAN
KUSS	Knut	Spectrum Laboratories Inc.	Breda	THE NETHERLANDS
KUSSER	Wolfgang	Tap Biosystems	Royston	UNITED KINGDOM
KYRIAKOPOULOS	Sarantos	Imperial College London	London	UNITED KINGDOM
KYUNG	Yun seung	Agensys, Inc. (An Affiliate Of Astellas Pharma Inc)	Santa Monica	UNITED STATES OF AMERICA
LAIDLAW	Dave	Kuhner Shaker Inc.	San Carlos	UNITED STATES OF AMERICA
LAIRD	Michael	Genentech, Inc.	South San Francisco	UNITED STATES OF AMERICA
LAM	Palmer	Biomarin Pharmaceuticals	Novato	UNITED STATES OF AMERICA
LAM	Betty	Biomarin Pharmaceuticals	Novato	UNITED STATES OF AMERICA
LAMBECK	Iris	Celonic Ag	Basel	SWITZERLAND
LANDAUER	Karlheinz	Celonic Ag	Basel	SWITZERLAND
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LANGE	Ingrid	Cobra Biologics	Södertälje	SWEDEN
LANGER	Uwe	Bayer Pharma Ag	Wuppertal	GERMANY
LAU	Carsten	Thermo Scientific	Lafayette	UNITED STATES OF AMERICA
LAU	Eduardo	University College London	London	UNITED KINGDOM
LAUX	Holger	Novartis Institutes For Biomedical Research	Basel	SWITZERLAND
LEBARBIER	Sylvain	Sartorius Stedim Biotech S.A	Aubagne	FRANCE
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LEE	Matthew	Aber Instruments Ltd	Aberystwyth	UNITED KINGDOM
LEE	Sojeong	Kaist	Daejeon	KOREA
LEE	Gyun min	Kaist	Daejeon	KOREA
LEE	Eun gyo	KRIBB	Daejeon	KOREA
LEE	Kyoungho	Osaka University	Osaka	JAPAN
LEE	Brian	Pbs Biotech, Inc.	Camarillo	UNITED STATES OF AMERICA
LEE	Kelvin	University Of Delaware	Newark, De	UNITED STATES OF AMERICA
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LIN	Nan	SAFC/Sigma-Aldrich	St. Louis	UNITED STATES OF AMERICA
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LOWAGIE	Serge	Proxylab Sprl	Beloeil	BELGIUM
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MAHAWORASILPA	Tohsak	The Stephen Sanig Research Institute	Eveleigh, Sydney	AUSTRALIA
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MALYGINA	Tamara	Optec	Novosibirsk	RUSSIA
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MARC	Annie	Cnrs	Vandœuvre-Les-Nancy	FRANCE
MARCEAU	Nicolas	Généthon	Evry	FRANCE
MARIGLIANO	Martine	Transgene Sa	Illkirch-Graffenstaden	FRANCE
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MARSH	Douglas	UCL Eli Lilly	London	UNITED KINGDOM
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MARTIN	Jessica	Sartorius Stedim North America Inc.	Bohemia	UNITED STATES OF AMERICA
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PERELMUTER	Karen	Institut Pasteur De Montevideo	Montevideo	URUGUAY
PÉRON	Gaël	Charter Medical, Ltd.	Melrand	FRANCE
PESCHANSKI	Marc	I-Stem / Inserm U861	Evry	FRANCE
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SCHOLZ	Jochen	Sartorius Stedim Biotech Gmbh	Göttingen	GERMANY
SCHRADER	Marion	Rentschler Biotechnologie Gmbh	Laupheim	GERMANY
SCHRÖDER	Petra	Eufets Gmbh	Idar-Oberstein	GERMANY
SCHUBEL	Andreas	Bayer Pharma Ag	Wuppertal	GERMANY
SCHUCHT	Roland	Inscreenex Gmbh	Braunschweig	GERMANY
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SEREDKINA	Olga	Optec	Novosibirsk	RUSSIA
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SEVCIKOVA	Jirina	/	Sardice	CZECH REPUBLIC
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SLADE	Ian	Genetic Engineering & Biotechnology News	New Rochelle	UNITED STATES OF AMERICA
SMALES	Mark	University Of Kent	Canterbury	UNITED KINGDOM
SMITH	Rodney	Macro Innovations Ltd	Cambridge	UNITED KINGDOM
SMITH	Shawn	Pall Life Sciences	Port Washington	UNITED STATES OF AMERICA

LAST NAME	FIRST NAME	COMPANY/INSTITUTE	CITY	COUNTRY
SOARES	Hugo	IBET-Instituto De Biologia Experimental E Tecnológica	Oeiras	PORTUGAL
SOM	Nathalie	Spinnovation Biologics	Nijmegen	THE NETHERLANDS
SOMMEREGGER	Wolfgang	Vienna Institute Of Biotechnology	Vienna	AUSTRIA
SOURABIE	Alain	Biospringer	Maison Alfort	FRANCE
SOUSA	Marcos	IBET-Instituto De Biologia Experimental E Tecnológica	Oeiras	PORTUGAL
SPIERINGS	Louis	IUL Instruments Gmbh	Königswinter	GERMANY
STAGGERT	Jill	Thermo Fisher Scientific	Lafayette	UNITED STATES OF AMERICA
STAHNKE	Bettina	Insilico Biotechnology Ag	Stuttgart	GERMANY
STAVELY	Adrian	Ge Healthcare	Little Chalfont	UNITED KINGDOM
STEFANI	Franck	Bioreliance	Glasgow	UNITED KINGDOM
STEINHARDT	Peter	Roche	Burgess Hill	UNITED KINGDOM
STEINHILBER	Silke	Ge Healthcare	Munchen	GERMANY
STERLING	John	Genetic Engineering & Biotechnology News	New Rochelle	UNITED STATES OF AMERICA
STETTLER	Matthieu	Merck Serono	Fenil Sur Cosier	SWITZERLAND
STEVENSON	Cory	Thermo Scientific	Lafayette	UNITED STATES OF AMERICA
STIEFEL	Fabian	University Of Applied Sciences Biberach	Biberach	GERMANY
STOBBE	Per	Cercell	Holte	DENMARK
STREEFKERK	Marcel	Dasgip Gmbh - An Eppendorf Company	Juelich	GERMANY
STREEFLAND	Mathieu	Wageningen University	Wageningen	THE NETHERLANDS
STROTBEK	Michaela	University Of Stuttgart	Stuttgart	GERMANY
STUETZLE	Martina	Institute Of Applied Biotechnology	Biberach A. D. Riss	GERMANY
SUBASHI	Ann	/	Mystic	UNITED STATES OF AMERICA
SUBASHI	Timothy	Pfizer	Groton	UNITED STATES OF AMERICA
SUZUKI	Yoko	University Of Fukui	Fukui	JAPAN
SVANHOLM	Bent	Svanholm.Com	Vordingborg	DENMARK
TABUCHI	Hisahiro	Chugai Pharmaceutical Co., Ltd.	Tokyo	JAPAN
TAKORS	Ralf	Institute Of Biochemical Engineering	Stuttgart	GERMANY
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TAPPE	Alexander	Sartorius Stedim Biotech Gmbh	Goettingen	GERMANY
TARDIEU	Fabienne	Molecular Devices	Wokingham	UNITED KINGDOM
TAYLOR	Charl	/	Camira	AUSTRALIA
TAYLOR	Karin	Australian Institute For Bioengineering And Nanotechnology	Brisbane	AUSTRALIA
TAYMANS	Didier	Glaxosmithkline Vaccine	Rixensart	BELGIUM
TEIXEIRA	Ana	IBET-Instituto De Biologia Experimental E Tecnológica	Oeiras	PORTUGAL
TERADA	Satoshi	University Of Fukui	Fukui	JAPAN
TESHIMA	Sumito	Japan Blood Products Organization	Kobe-Shi, Hyogo	JAPAN
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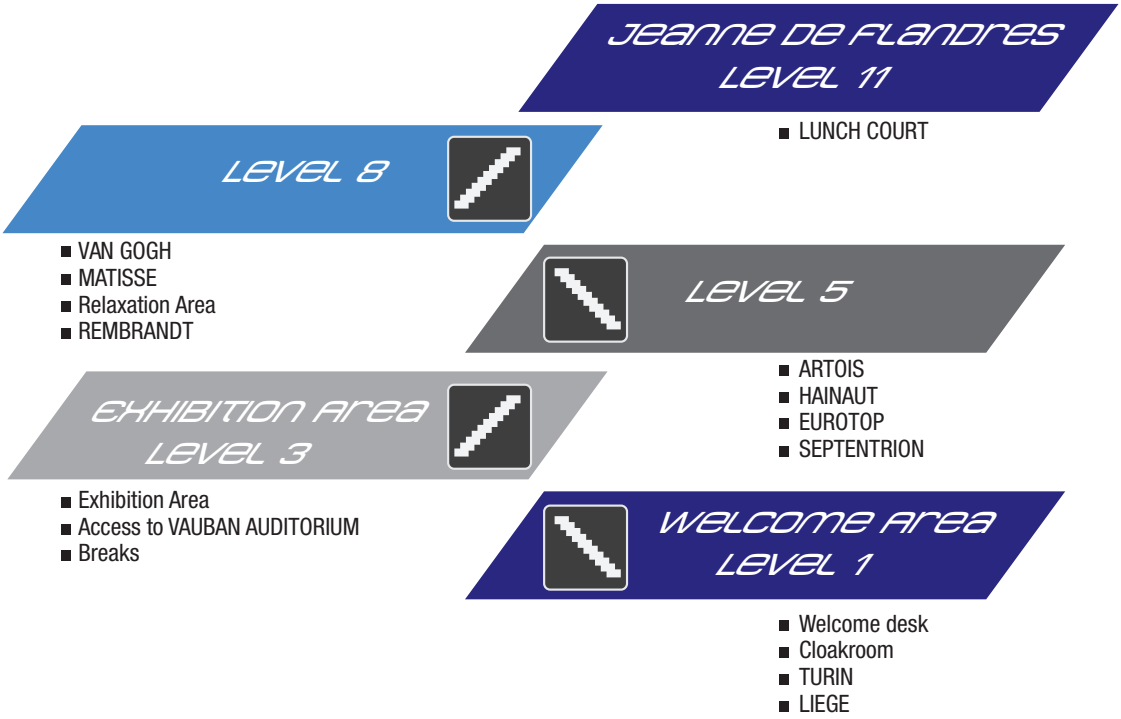
THIÉBART	Delphine	Global Process Concept	La Rochelle	FRANCE
THIELE	Michael	Probiogen Ag	Berlin	GERMANY
THOMPSON	Christine	National Research Council	Montreal	CANADA
THOMSEN	Henrik m.	Chemometec A/S	Allerod	DENMARK
THOMSON	Vicky	Thermo Scientific	Lafayette	UNITED STATES OF AMERICA
THRIFT	John	Bayer Haelthcare	Berkeley	UNITED STATES OF AMERICA
TILLICH	Dirk	Finesse	Santa Clara	UNITED STATES OF AMERICA
TILLMAN	Ulrich	Merck Kgaa	Darmstadt	GERMANY
TIMMERMANN	Christina	University Bielefeld	Bielefeld	GERMANY
TINGLEY	Stephen	Repligen Corporation	Waltham	UNITED STATES OF AMERICA
TINTRUP	Hartmut	Cevec Pharmaceuticals	Cologne	GERMANY
TOLSTRUP	Anne bondgaard	Bl Pharma Gmbh & Co. Kg	Biberach An Der Riss	GERMANY
TONSO	Aldo	Universidade De São Paulo	São Paulo	BRAZIL
TRAMNITZ	Birgit	Medicyte Gmbh	Heidelberg	GERMANY
TRÄNKLE	Jens	Bayer Technology Services Gmbh	Leverkusen	GERMANY
TRÅSDAHL HAUGSETH	Kirsten	Pharmaq	Oslo	NORWAY
TRÖBS	Thomas	Roche Diagnostics Gmbh	Penzberg	GERMANY
TSURUNARI	Moe	Jx Nippon Oil & Energy Corporation	Toda, Saitama	JAPAN
TUNG	Meg	Genentech, Inc.	South San Francisco	UNITED STATES OF AMERICA
TYACKE	Sean	Roche Diagnostics	Burgess Hill	UNITED KINGDOM
ULLAH	Millie	/	Langenselbold	GERMANY
USAKU	Chonlatep	Imperial College London	London	UNITED KINGDOM
VAESSEN	Erik	Irvine Scientific	Santa Ana	UNITED STATES OF AMERICA
VALLÉE	Cédric	École Polytechnique De Montréal	Montréal	CANADA
VAN DEN BERG	Hans	Finesse	Santa Clara	UNITED STATES OF AMERICA
VAN DER AAR	Pim	Dync B.V.	Breda	THE NETHERLANDS
VAN DORSTEN	Ferdi	Spinnovation Biologics	Nijmegen	THE NETHERLANDS
VAN OPSTAELE	Nadine	Selborne Biological Services	Alton Hampsire	UNITED KINGDOM
VAN TRIER	Mark	Jm Separations Bv	Tilburg	THE NETHERLANDS
VAXELAIRE	Emilie	Glenmark Pharmaceuticals Sa	La Chaux-De-Fonds	SWITZERLAND
VÁZQUEZ RAMÍREZ	Daniel	Max Planck Institute For Dynamics Of Complex Technical Systems	Magdeburg	GERMANY
VEITH	Nathalie	Fraunhofer Item	Braunschweig	GERMANY
VELNOSKEY	Chuck	BD Biosciences	Sparks	UNITED STATES OF AMERICA
VENTINI MONTEIRO	Daniella	Instituto Butantan	São Paulo	BRAZIL
VERFAILLIE	Catherine	Ku Leuven - Stamcelinstituut	Leuven	BELGIUM
VILLACRÉS BARRAGÁN	Ana carina	University Of Manitoba	Winnipeg	CANADA

LAST NAME	FIRST NAME	COMPANY/INSTITUTE	CITY	COUNTRY
VILLAREAL	Myra	University Of Tsukuba	Tsukuba City	JAPAN
VINCENT	Simon	SAFC	Irvine	UNITED KINGDOM
VISIER	Jean luc	Cellon	Luxembourg	LUXEMBOURG
VLEUGELS	Mieke	BD Biosciences	Erembodegem	BELGIUM
VOEDISCH	Bernd	Novartis Institutes For Biomedical Research	Basel	SWITZERLAND
VOLDBORG	Bjørn	Technical University Of Denmark	Hoersholm	DENMARK
VON HAGEN	Joerg	Merck Kgaa	Darmstadt	GERMANY
VON STRANDMANN	Ralph	Tap Biosystems	Royston	UNITED KINGDOM
VONACH	Bénédicte	Novartis Pharma	Basel	SWITZERLAND
WADAAN	Mohammad	King Saud University, Faculty Of Science	Riyadh	SAUDI ARABIA
WAGNER	Roland	Rentschler Biotechnologie Gmbh	Laupheim	GERMANY
WAGNER	Sven	Sartorius Stedim Biotech Gmbh	Göttingen	GERMANY
WAGNER	Ilka	Tu Berlin	Berlin	GERMANY
WAGUER	Ruth	Octapharma	Neidelberg	GERMANY
WAHAB	Omar	SAFC	Gravesend	UNITED KINGDOM
WAHRHEIT	Judith	Universität Des Saarlandes	Saarbrücken	GERMANY
WALKER	Matthew	Tap Biosystems	Royston	UNITED KINGDOM
WARD	Sally	UTSouthwestern Medical Center	Dallas	UNITED STATES OF AMERICA
WARKE	Vishal	Himedia	Mumbai	INDIA
WARR	Steve	Glaxosmithkline	Stevenage	UNITED KINGDOM
WARSCHKAU	Holger	BD Biosciences	Berlin	GERMANY
WATTENBERG	Andreas	Protagen Protein Services Gmbh	Dortmund	GERMANY
WATTRE	Fabien	Infors	Massy	FRANCE
WEBER	Christian	Merck Millipore	Darmstadt	GERMANY
WEHLMANN	Hermann	Bayer Pharma Ag	Wuppertal	GERMANY
WEICHERT	Henry	Sartorius Stedim Biotech Gmbh	Göttingen	GERMANY
WEISS	Bettina	University Of Applied Sciences	Esslingen	GERMANY
WELSINK	Tim	Invivo Biotech Services Gmbh	Hennigsdorf	GERMANY
WENDEROTT	Alexandra	Dasgip Gmbh - An Eppendorf Company	Juelich	GERMANY
WENDT	Maria	Genedata	Basel	SWITZERLAND
WÉRENNE	John	Université Libre De Bruxelles	Brussels	BELGIUM
WERNER	Sören	Zurich University Of Applied Sciences	Wädenswil	SWITZERLAND
WEST	Larry	Aspen Brook Consulting, Inc	Park City, Ut.	UNITED STATES OF AMERICA
WHELAN	Jessic	Applied Process Company (Apc)	Dublin	IRELAND
WHITFORD	William	Thermo Fisher Scientific	Lafayette	UNITED STATES OF AMERICA
WIEDEMANN	Philipp	Mannheim University Of Applied Sciences	Mannheim	GERMANY
WIELAND	Daniel	Ge Healthcare	Munchen	GERMANY
WILCOX	Christopher	Kerry Inc.	Beloit	UNITED STATES OF AMERICA
WILTBERGER	Kelly	Biogen Idec	Durham	UNITED STATES OF AMERICA
WINKLER	Karsten	Probiogen Ag	Berlin	GERMANY
WINTER	Jacques	Infors Sarl	Massy	FRANCE
WINTGENS	Marc	Selborne Biological Services	Alton Hampsire	UNITED KINGDOM
WIPPERMANN	Anna	Bielefeld University	Bielefeld	GERMANY
WIRTH	Dagmar	Helmholtz Centre For Infection Research	Braunschweig	GERMANY
WISSING	Silke	Cevec Pharmaceuticals	Köln	GERMANY
WÖLFEL	Jens	Cevec Pharmaceuticals Gmbh	Cologne	GERMANY
WOOD	Amy	Emd Millipore Corporation	Bedford	UNITED STATES OF AMERICA
WRIGHT	Chapman	Biogen Idec	Cambridge	UNITED STATES OF AMERICA
WURM	Florian m.	Swiss Federal Institute Of Technology Lausanne	Lausanne	SWITZERLAND
WYNNE	John	Merck Millipore	Knutsford	UNITED KINGDOM
YAMAJI	Hideki	Kobe University	Kobe	JAPAN
YAMAUCHI	Rinaka	University Of Fukui	Fukui	JAPAN
YE	Haifeng	Eth Zurich	Basel	SWITZERLAND
YOKOTA	Masami	Astellas Pharma Inc.	Tsukuba-Shi, Ibaraki	JAPAN
YOSHIMOTO	Nobuo	/	Nagoya	JAPAN
YOUNG	Robert	Lonza Biologics Plc	Cambridge	UNITED KINGDOM
YOUNG	Don	Thermo Scientific	Lafayette	UNITED STATES OF AMERICA
YUSUFI	Faraaz	Bioprocessing Technology Institue, A*Star	Singapore	SINGAPORE
ZENG	Steffen	Boehringer Ingelheim Pharma Gmbh & Co. Kg	Biberach	GERMANY
ZHANG	Min	Fujifilm Diosynth Biotechnologies	Cary	UNITED STATES OF AMERICA
ZHANG	Lin	Pfizer	Andover	UNITED STATES OF AMERICA
ZHANG	Ye	Royal Institute Of Technology, Sweden	Stockholm	SWEDEN
ZIJLSTRA	Gerben	Dsm Biologics	Groningen	THE NETHERLANDS
ZIJLSTRA	Dirk	Enzyscreen Bv	Haarlem	THE NETHERLANDS
ZIMMER	Aline	Merck Kgaa	Darmstadt	GERMANY
ZOTTER	Angelika	Austrian Center Of Industrial Biotechnology	Vienna	AUSTRIA
ZUMPE	Stefanie	Bioengineering Ag	Wald	SWITZERLAND
ZUMSTEIN	Joel	Novartis Pharma Ag	Basel	SWITZERLAND

LILLE GRAND PALAIS MAP



PROGRAMME AT A GLANCE

	Sunday 23rd June		Monday 24th June		Tuesday 25th June		Wednesday 26th June
9h00	Workshops	9h00	Session II	9h00	Session IV	9h00	Session V
10h00		10h00	Coffee Break	10h00		10h00	
11h00	Coffee Break	11h00	Session II	11h00	Coffee Break	11h00	Coffee Break
12h00	Workshops	12h00		12h00	Session IV	12h00	Session VI
13h00	Lunch Break	13h00	Lunch and Poster Session A	13h00	Lunch and Poster Session B / ESACT GENERAL ASSEMBLY	13h00	Lunch and Poster Session C
14h00	Workshops	14h00		14h00		14h00	
15h00 15h45		15h00	Session III	15h00	Outing in Lille and cities around	15h00	Keynote Lecture : C. VERFAILLIE
16h00	Opening ceremony Keynote Lecture : A. CAPLAN	16h00	Coffee Break	16h00		16h00	Short Presentation & Poster Prize Session
17h00	Session I	17h00	Session III	17h00		17h00	Closing ceremony
18h00		18h00		18h00		18h00	
19h00	Traders Reception	19h00		19h00	Dinner at the Museum of Fine Arts of Lille	19h00	Gala Dinner at the «Farmhouse of the Templars»
20h00		20h00		20h00		20h00	