CHAPTER23

Product and process design: towards industrial TE manufacturing

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Learning Objectives

- An understanding of the challenges that tissue engineered products will face at the translational phase
- Overview of the sequence of process steps involved in tissue engineering manufacturing
- Possible strategies to manufacture tissue engineered products
- How current technologies fit and can be best used for autologous and allogeneic tissue engineered products
- How to determine quality characteristics and link them to the production process environment
- Using monitoring and control for real time follow up of TE product maturation
- Understand the importance of models for the design of novel tissue engineered products and bioprocess optimization.
- What is downstream process in the case of TE manufacturing
- Integration of process steps in larger schemes, 'whole bioprocessing' approach

Abstract

As the field of Tissue Engineering matures and the transition from bench-scale to large-scale industrialized production is realized, a new set of biological and technological challenges arises. To bring tissue engineered products to the clinic and subsequently to the market will require the application of engineering principles and practices to achieve control, reproducibility, automation, validation and safety of the process and the product. The successful translation will require contributions from fundamental research (from

developmental biology to advanced modeling mathematical approaches) but also existing industrial practice (biopharma), especially on automation, quality assurance and regulation.

Keywords

Autologous and allogeneic, current good manufacturing practice (cGMP), Bioprocess development and optimization, Quality control, Product design, Downstream processing

Snapshot Summary

- The translation of tissue engineered products to the market will require a new set of technological challenges to be addressed. In order to achieve this there is a need for engineering principles to be applied to bring biologic discoveries to the market.
- There are two main categories of Tissue Engineered products based on the origin of the cells in the TE (i) allogeneic therapies may result in 'off-the-shelf' products ready to be administered to patients, (ii) autologous 'patient-specific' therapies based on the use of the patient's own cells for the production of the TE product.
- To produce TE products without risk of contamination, GMP grade raw materials and facilities should be the base for TE manufacturing.
- The production of TE products will consist on a series of process steps which need to be designed, understood and optimized. Currently there is lack of process characterization while manual operations are still dominant.
- Quality control for TE products should take into account the complexity of the product. A mutliscale approach characterizing cells but also tissue properties (3D) will be needed hence apart from standard techniques for cell characterization novel ones should be developed.
- For TE products the end product is linked directly to the process due to the responsiveness of the cultured cells to their environment. Monitoring and controlling the culture environment may be indirectly linked to the quality properties of the TE construct.
- Data derived from sensors should be analysed by algorithms, if possible, online to extract maximum information and aid in decision making during manufactruring

- Bioreactor systems can delivery sufficient volumes for industrial production of allogeneic TE products while providing control options and automation potential which is crucial for autologous treatments
- Downstream processes will be required for the harvest and recovery of cells, their purification and potentially re-seeding to scaffolds. Currently there is lack of information on this aspect of TE manufacturing however in a commercial setting this will become necessary
- Mathematical modeling may be applied for the design of TE constructs by running cost effectively in silico experiments. This can be applied both for in vitro as well as in vivo case studies. Models characterizing cell-scaffold interactions, cell signaling , tissue growth and in vivo behavior should eventually be linked across scales.

10 Suggested papers

1. Abbasalizadeh, S. Baharvand H. 2013. Technological progress and challenges towards cGMP manufacturing of human pluripotent stem cells based therapeutic products for allogeneic and autologous cell therapies. Biotechnology Advances*,* 31**,** 1600-1623.
2. Csaszar, E., Kirouac, D.C., Yu, M., Wang, W.J., Qiao, W.L., Cooke, M.P., Boitano, A.E., Ito, C., Zandstra. P.W. (2012). Rapid Expansion of Human Hematopoietic Stem Cells by Automated Control of Inhibitory Feedback Signaling. Cell Stem Cell, 10, 218-229.
3. Yeo, D., Kiparissides, A., Cha, J.M., Aguilar-Gallardo, C., Polak, J.M., Tsiridis, E., Pistikopoulos, E.N., Mantalaris, A. (2013). Improving Embryonic Stem Cell Expansion through the Combination of Perfusion and Bioprocess Model Design, PloS one.
4. Hunt, M.M., Meng, G., Rancourt, D.E., Gates, I.D., Kallos, M.S. (2014) Factorial experimental design for the culture of human embryonic stem cells as aggregates in stirred suspension bioreactors reveals the potential for interaction effects between bioprocess parameters. Tissue Eng Part C Methods, 20, 76-89.

5. Kinney, M.A., McDevitt, T.C. (2013). Emerging strategies for spatiotemporal control of stem cell fate and morphogenesis, Trends in biotechnology, 31, 78-84.
6. Chang, T., Zimmerley, M.S., Quinn, K.P., Lamarre-Jouenne, I., Kaplan, D.L., Beaurepaire, E., Georgakoudi, I. (2013). Non-invasive monitoring of cell metabolism and lipid production in 3d engineered human adipose tissues using label-free multiphoton microscopy. Biomaterials, 34, 8607–8616.
7. Lambrechts, D., Roeffaers, M., Kerckhofs, G., Roberts, S.J., Hofkens, J., Van de Putte, T., Van Oosterwyck, H., Schrooten, J. (2013). Fluorescent oxygen sensitive microbead incorporation for measuring oxygen tension in cell aggregates, Biomaterials, 34, 922-929.
8. Wu, J., Tzanakakis, S. (2013). Deconstructing stem cell population heterogeneity: Single cell analysis and modeling approaches. Biotechnology Advances, 31, 1047-1062
9. Diogo, M.M., da Silva, C.L., Cabral, J.M., (2012) Separation technologies for stem cell bioprocessing, Biotechnology and Bioengineering, 109,2699-2709.
10. Simaria, A.S., Hassan, S., Varadaraju, H., Rowley, J., Warren, K., Vanek, P., Farid, S.S. (2014) Allogeneic Cell Therapy Bioprocess Economics and Optimization: Single-Use Cell Expansion Technologies. Biotechnology and Bioengineering, 111, 69-83.

3.1 Introduction

The steady increase of early- and late-stage clinical trials involving cell therapy applications, as well as the presence of approved commercial tissue engineered (TE) products in the market, strongly indicates that the cell therapy industry is on its way to evolve into a novel healthcare sector (Mason et al., 2013, Mason and Manzotti, 2010). Apart from clinical efficacy, TE products will need to be manufactured in cost-effective, scalable and robust bioprocesses that at the same time also meet the requirements of regulatory bodies in terms of quality control (QC) and good manufacturing practice (GMP) (dos Santos et al., 2011, Ratcliffe et al., 2011). TE product manufacturing will require a series of process steps from biopsy harvest and stem cell isolation for autologous strategies, or starting from master cell bank frozen vials for allogeneic strategies, to end product formulation and implantation. Currently, stem and progenitor cell isolation, expansion, differentiation and 3D tissue construct formation consist of a series of conventional manual and static techniques (Figure 1), heavily depending on operator expertise. Hence these process steps are still suboptimal and uncontrolled, with high risk of contamination and processing inconsistency (Mason and Hoare, 2007, Placzek et al., 2009).

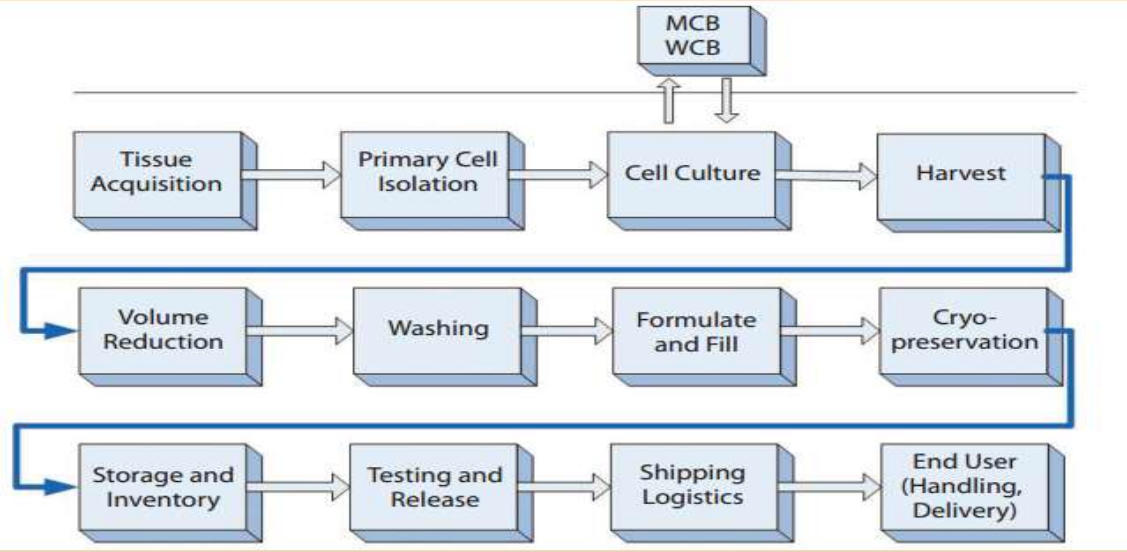

Figure 1: Sequence of units of operations involved in TE manufacturing. Processes from biopsy acquisition (or master cell bank (MCB)) through cell expansion, cell harvest condensation and formulation are all components of the pipeline that will be required for cell and tissue engineered construct production.

In addition, manual operations are an additional source of complexity to TE construct production, where the fusion of numerous biological 'raw' materials is required to manufacture complex final products. Furthermore end product quality attribute profile (such as cell number, phenotype, extracellular matrix content/morphology) will be constantly affected by the bioprocess environment. Hence the translation of laboratory-scale produced three dimensional (3D) engineered constructs to clinically effective and economically viable products requires the development of efficient and robust manufacturing processes that will ensure consistent product quality according to regulatory requirements addressing key barriers to translation (French et al., 2014). An increasing number of recent review papers is highlighting the need for a concerted effort, between academic, clinical, regulatory and industrial partners in order to outline the landscape for research and translation suggesting a global standardization and harmonization (ESF how to advance cell-based Advanced Therapies in Europe: http://www.esf.org/index.php?eID=tx_nawsecuredl&u=0&file=fileadmin/be_user/research_areas/emrc/RNPs/Remedic/2013/White_paper_REMEDIC_22_July_2013_final_v1.pdf&t=1396269348&hash=e5e0dbdbaa744fed8a1123d88adbe12571469553). This global attempt should follow rational and orchestrated approaches rather than trial and error ones, from stem cell niche to process-scale (Kirouac and Zandstra, 2008), taking into account the development of commercially viable and robust bioprocesses that will ultimately allow for a cost effective production of TE products (http://scec.gatech.edu/sites/default/files/StemCellEngineering-CompleteReport.pdf), (Nerem, 2012).

23.1.2 Satisfying cGMP requirements

cGMP regulations were first introduced by the US Food and Drug Administration and are also implemented in Europe (Commission Directive 2003/94/EC). cGMP regulations aim to ensure the identity, quality, safety, purity and potency of TE products. (FDA cGMP guidelines,http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064971.htm). All clinical trials currently in the U.S. are obliged to produce all cell products following FDA cGMP guidelines (Code 21 CFR 210–211, 312, 600, and 1271). Equivalent regulatory rules have also been established in Europe (EU Directive 2003/94/EC) and many other countries and regions internationally (ICHQ7). To achieve this, all processing

stages should adhere to the guidelines by using cGMP-grade reagents and materials as well as cGMP-compliant systems that operate under current cGMP conditions. Besides the manufacturing process itself, also a quality management system that can precisely monitor, measure, and record critical quality parameters in each processing step is required. This quality management systems need to ensure that the final TE product is manufactured according to the desired safety, quality, quantity, purity, and potency requirements of the targeted clinical application. Hence to obtain effective and clinically relevant cell-based therapeutic products manufacturing in stringent and reliable context is needed. More specifically for TE product manufacturing all 'raw' materials, like biomaterials, substrates, culture media or biomolecules (growth factors) that create an adequate environment for the propagation of stem cells in an undifferentiated state and/or their controlled differentiation into more mature cells, should (at the start of the process) individually all satisfy cGMP requirements.

cGMP requirements were established to be flexible in order to allow each manufacturer to decide individually how to best implement the necessary controls by using scientifically sound design, processing methods, and testing procedures. The "c" in cGMP stands for "current," meaning that technologies and systems that are up-to-date should be employed in order to comply with the existing regulations. However given the complexity and range of the expected TE products a variety of customized 'rules' would be required at least at the early phases to conduct a product-based characterization and therefore it should be noted that cGMPs are only minimum requirements. Approved cell-based advanced therapeutic medicinal products (ATMPs) such as MACI (matrix-induced autologous chondrocyte implantation), aiming to repair cartilage defects (Genzyme/Sanofi, France) and ChondroCelect® (TiGenix, Belgium) are the only licensed cell-based ATMPs on the market in Europe and could provide influential blueprints for their cGMP procedures (Warren, 2013).

TE	Tissue Engineering
ATMP	Advanced therapeutic medicinal product
EMA	European medicines agency
FDA	Food and Drug administration
cGMP	Current good manufacturing practice
QbD	Quality by Design

DoE	Design of experiments
CQA	Critical quality attribute
CPP	Critical process parameter

Table 1: Abbreviations for commonly used terms related to Tissue Engineering manufacturing

23.1.3 Quality by design: pathway for optimized translational strategies

Quality by design (QbD) is a systematic approach to drive development that begins with **predefined objectives** and emphasizes product and process understanding and process control, based on sound science and quality risk management. The application of the QbD principles in TE bioprocess development has been brought forward as a strategy to ensure that manufactured TE products will possess predefined quality characteristics addressing emerging regulatory requirements and ensuring a functional in vivo behavior, facilitating thus the road to clinical use and commercialization (Rathore and Winkle, 2009). Primary QbD considerations that need to be taken into account while designing bioprocesses should focus on: (i) the materials to be used (process components), (ii) the various manipulations and assessments of the materials (process requirements), and (iii) the performance of the output product(s) (process function), for TE products, regenerative potential upon implantation (Figure 2).

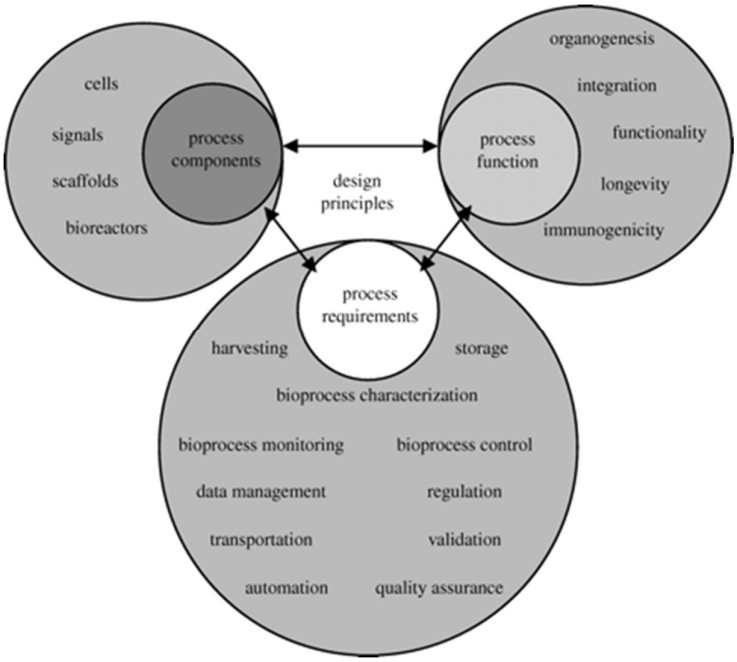

Figure 2: Design principles for stem cell and tissue engineering bioprocessing (Placzek et al., (2009). J. R. Soc. Interface **6,**209-232.).

Understanding and refining of the manufacturing process, includes:

- To explore and identify, the material attributes and process parameters that can have an effect on final TE product **critical quality attributes (CQAs)**
- To determine functional relationships that link **critical quality attributes** to critical **process parameters (CPP)**

This means that out of a range of final quality characteristics of a TE product (quality target profile) only a subset may be linked to its efficacy and therapeutic potential, and should be therefore controlled and built in to during its manufacturing. This should be carried out for each process step involved in the manufacturing of TE products. For instance homogeneity, during the scaffold seeding process step, may be a more important quality attribute than the seeded cell number for the therapeutic value of the TE construct, and therefore should be termed a CQA. This means that the manufacturer should guarantee homogeneity prior to maximizing cell presence. It should be noted that the quality attributes of upstream processes will affect the performance of subsequent processes but also end-product quality profile and its performance *in vivo*. Those TE product quality attributes that will result in the desirable in vivo behavior should be identified and included in the optimization loop.

One of the most important concepts in QbD is termed as 'design space' and its definition was given by the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use ((ICH) Q8 guidance document), as ''the multidimensional combination and interaction of input variables that have been demonstrated to provide an assurance of quality'' (FDA, 2006, 2009). So

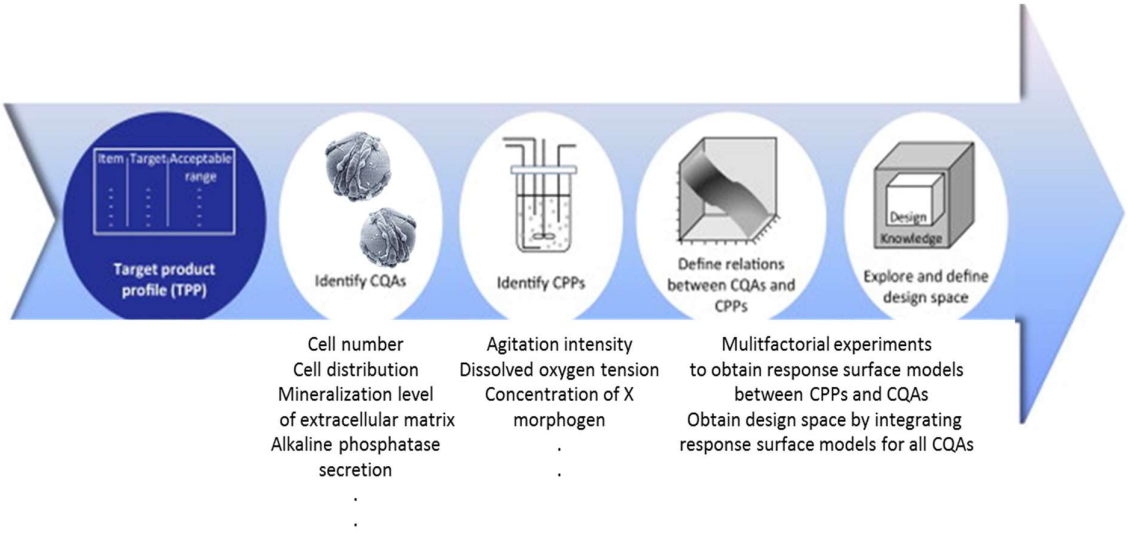

Figure 3: Typical approach for QbD implementation in bioprocess development and the role of multifactorial design of experiments and analysis. A case study for bone tissue engineering is shown. (Figure adapted from Mercier et al. (2014) Trends in Biotechnology. http://dx.doi.org/10.1016/j.tibtech.2014.03.008).

Multifactorial design of experiments (DoE) is a component of QbD strategies and has been suggested as an important tool to develop efficient procedures that enable multiple factor experiments. As TE process and products intrinsically consist of a multitude of variables, DoE allows data to be obtained in such a way that it yields consistent and objective conclusions on the involvement and statistical significance of all investigated parameters but also of their interactions. Hence process parameters can be linked with TE product quality attributes via statistical correlations named response surfaces. Furthermore, DoE requires a smaller number of experimental runs, while, at the same time, covering a broader knowledge space than a one factor at a time approach. DoE has been used for optimizing complex medium compositions for stem cell culture (Lim et al., 2012). Multifactorial DoE was employed to investigate the effect of inoculation (seeding) density and agitation rate for stirred suspension bioreactors while expanding hESC aggregates, Significant interaction effects between inoculation density and agitation rate specifically in the case of exponential growth rates was observed. This study showed that a stepwise optimization may result in missing out on the real optimal operation regime (Hunt et al., 2014).

23.1.2 Allogeneic vs autologous strategies

TE therapies are often divided into two main categories: 'patient-specific'/autologous and 'off-the-shelf'/allogeneic. (Wang et al., 2013a). Pioneering cell therapies (e.g. blood cell transfusions, bone marrow transplantation) were 'patient-specific', due to the need for histocompatibility matching. More recent therapies, however, are based in cell types that are low immunogenic (e.g. MSCs) and could therefore be more amenable to the development of 'off-the-shelf' products, more similar to traditional biopharmaceutical products (see 23.1.2). An 'off-the-shelf' cell therapy product is likely to be mass produced in large-scale processes, taking advantage of bioprocess technology and also efficient scale-up strategies. The manufacturing process usually involves the establishment of master and working cell banks (facilities that stores cells of specific genome for the purpose of future use in a product or medicinal needs) that will then be used for the production of large numbers of cells for subsequent processing. Cells will need to go through a method of isolation and banking, followed by bioprocessing (expansion, controlled differentiation and sorting) in a centralized manufacturing facility to produce the cell and neotissue type of choice for clinical use (Figure. 4). In contrast, 'patient-specific' therapies are based in autologous strategies or in tight histocompatibility matching and thus production is likely to be performed on an individual basis (Figure 4).

Each of these approaches will likely require different solutions in terms of bioprocessing. Scalability can be implemented either in volume based metrics (scale-up) or in unit based metric (scale-out). 'Off-the-shelf' therapeutic products will most likely be manufactured based on a 'scale-up' strategy, which consists in the development and optimization of cell processes that begin at a small scale while subsequently, progressively increase the volume of the system, while maintaining the efficiency of the process. This approach, however, may not be appropriate for 'patient-specific' therapies, which will have to be 'scaled-out' in tightly controlled bioprocesses. This means that the process will have to be extensively replicated to increase the final cell number due to the large number of strictly parallel culture events. Also cell performance unpredictability across this parallel processing will have to be taken into account due to 'donor to donor' variability. However in the context of 'allogeneic' Tissue Engineering a combined approach composed of an initial step aiming at scale-up for cell expansion, followed by a 'scaled-out' perfusion culture for subsequent differentiation stages or 'neotissue' formation, could be followed.

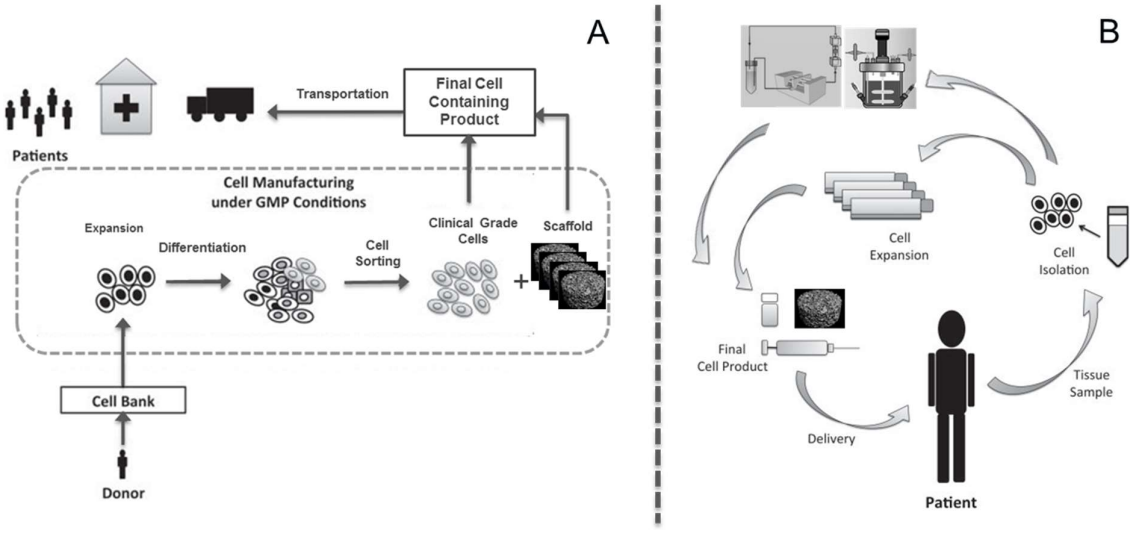

Figure 4: (A) Flow chart for the production of an 'off-the-shelf' cell therapy product. Allogeneic treatments are likely to be mass-produced using large-scale bioprocessing. (B) Flow chart for the production of a 'patient-specific' cell therapy product. Such therapies are based in autologous strategies or in tight histocompatibility matching, and thus production is likely to be performed on an individual basis. (Adapted from Fernandes et al. Stem cell bioprocessing for regenerative medicine. J Chem Technol Biotechnol 2014; **89**: 34–47).

23.2 Bioreactor systems for TE product manufacturing

Bioreactors as already discussed, will play a crucial role for TE product development. They provide the means to overcome limitations of conventional manual methods by (i) delivering sufficient cell numbers for multiple doses per batch and hence provide scale up potential (Yeatts and Fisher, 2011, Zweigerdt et al., 2011, Wang et al., 2013b) (ii) helping to develop structurally defined and functionally effective complex 3D engineered constructs at the patient-scale using scale-out strategies(Wendt et al., 2009, Salter et al., 2012). The ability to develop automated bioreactor systems with steady-state control of bioprocess parameters such as flow rate/shear stress, dissolved oxygen tension, pressure drop (for perfusion bioreactor systems) and nutrient supply minimizes complexity and variability of the environment that the cells will experience (Martin et al., 2009, dos Santos et al., 2013). At present commercial platforms that succeeded in automating 2D cell culture such as Fraunhofer's – 'The Tissue Factory' (http://www.tissue-factory.com/en/The_Tissue_Factory.html) are available, showing the maturation of the field. However, automation will ultimately rely on information provided by the use of closed bioreactor systems that incorporate non-invasive online monitoring modalities. These

modalities will allow the follow up of the quality characteristics of manufactured ATMPs during stem cell culture. Reproducible bioprocesses that will deliver the quantity and quality of the required cellular and tissue product should be the cornerstone for successful translation strategies (Yeo et al., 2013).

23.2.1 Bioreactors for allogeneic strategies

Allogeneic products will most likely be produced in large quantities and will therefore require suspension culture systems that will allow for scale-up in order to meet the demand. Bioreactor platforms available for producing adherent cells — including planar technologies, packed-bed systems, and suspension platforms such as microcarriers and aggregate cultures — have been employed for their potential to satisfy batch requirements at different scales. Microcarrier based bioreactor systems such as stirred tanks (Chen et al., 2013b, dos Santos et al., 2011) and spinner flasks (stirred suspension bioreactors) (dos Santos et al., 2011) and also wave bags (Timmins et al., 2012) have been successfully used for human mesenchymal stem cell expansion (MSC). Recently a number of studies employed xeno-free components for the expansion of stromal MSCs as well as for human pluripotent cells (hPS) cells (Fan et al., 2014) and induced pluripotent cells (iPS) cells (Wang et al., 2013b) showing alignment with GMP requirements. Cell expansion using 3D aggregates has been also achieved in shake flasks for MSC (Frith et al., 2010) and hPS cells (Abbasalizadeh et al., 2012b) and in hollow fiber systems (Nold et al., 2013) with the retention of their differentiation potential (Figure 3). Furthermore commercial stem cell expansion systems already exist. These platforms, like the Terumo Quantum® hollow fiber and the single-use Integrity Xpansion™ multiplate bioreactor system (see Figure 5), provide a GMP platform with enhanced reproducibility and scalability.

Type	Advantages	Disadvantages	Scale-up / Scale-out
Stirred suspension	Homogeneity in culture environment due to mixing. Allows cell-cell interactions.	Excessive agglomeration between aggregates/microcarriers. Sensor readouts may prove inaccurate due to diffusion within 3D aggregates	Scale up
Wave bag	Disposable minimizes	Cost.	Scale up

	contamination risks. Easy to scale up	Control of process parameters.	
Fixed bed / perfusion	Allows cell - cell and cell-ECM interactions. Incorporation of bioactive scaffolds. Direct control over shear stress development. Accurate sensor read outs at the outlet of the bioreactors.	Low volume Spatial gradient build-up. No sampling of cells is possible during culture. Difficulties in cell harvest	Scale out
Hollow fibre	Easy to monitor and control. Low shear stress.	Gradient build up at the hollow fibre interface. 3D cell growth may alter flow patterns within the hollow fibres.	Scale out
Multiplate	Allows image follow up. Close to the low risk commonly used paradigm of the T-flask.	Non physiological cell culture environment Difficulties in cell harvest	Scale up

Table 3: Bioreactor systems for cell culture advantages disadvantages role in the TE manufacturing pipeline

23.2.2 Bioreactors for autologous strategies

As the reintroduction of cells to the patient will require a rapid in vitro stage prior to implantation, tightly controlled systems will providing reliable monitoring potential should be used. For the cells-seeded-on-3D-scaffold paradigm the flow-through perfusion bioreactor is the reactor of choice (Marolt et al., 2012). Upon cell attachment to the carrier/scaffold, packed-bed perfusion systems provide continuous control options that overcome the limitations of traditional, standard culture methodologies. A broad range of variations on these bioreactor systems has been described to date in literature for the production of three dimensional neotissue constructs (Frohlich et al., 2010, Grayson et al., 2010, Papantoniou et al., 2012, Kim and Ma, 2012). For example bone repair applications via the intramembranous (Yeatts et al., 2014, Janssen et al., 2010) as well as endochondral bone forming (Scotti et al., 2013) pathway have been reported upon implantation of human mesenchymal stem cells expanded in perfusion bioreactors in animal models

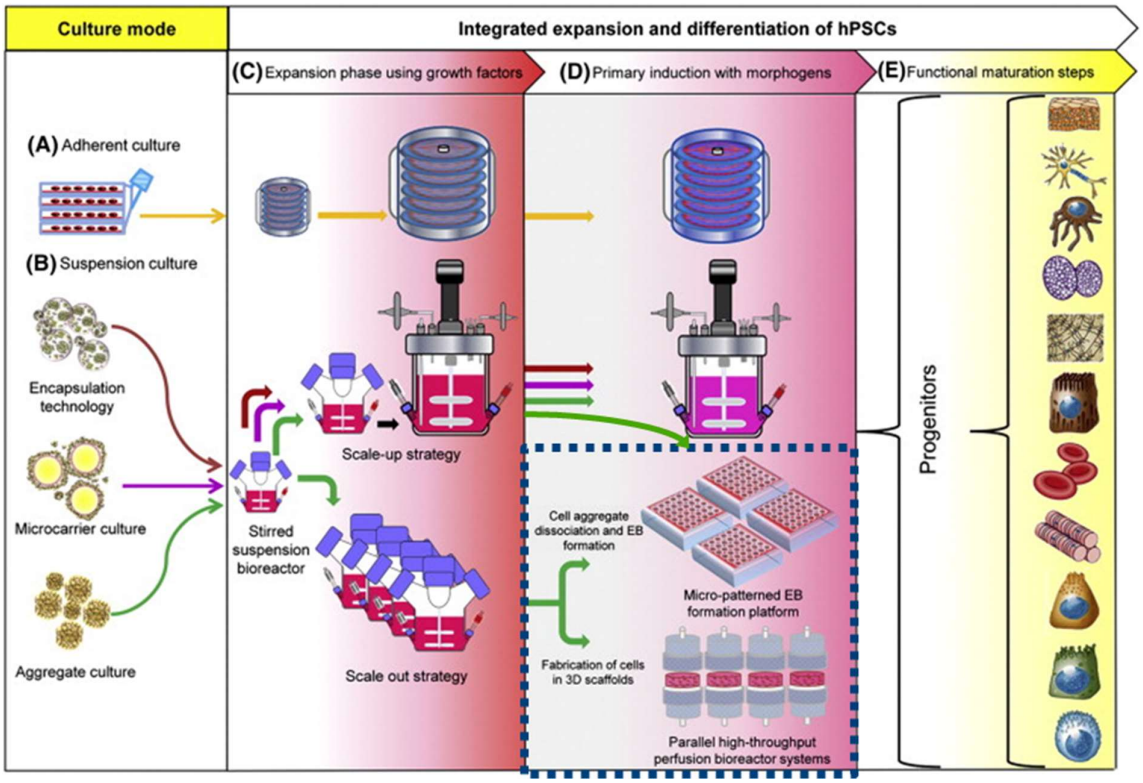

Figure 5: Scalable platforms for integrated expansion and differentiation of hPSCs including integrated expansion and differentiation of hPSCs in dynamic adherent culture systems (A) and suspension culture systems (B) comprising encapsulation technology, microcarrier based cultures, and aggregate culture in stirred suspension bioreactors. Differentiation could be initiated after reaching desired undifferentiated cell numbers (C) by replacing the expansion media with differentiation media for early differentiation in the same expansion culture system or dissociating the cell aggregates in aggregate culture systems and transferring the single cells to high-throughput perfusion systems or micro-patterned cell aggregates based on targeted differentiation (D) and then starting functional maturation steps using different morphogens to produce target progenitor or terminally differentiated cells (E). (From (Saeed A and Baharvand H, (2013) Biotechnology Advances, 31, 1600-23)).

23.3 Quality control for TE products – a multiscale approach

TE product characterization and analysis will be of higher complexity than for other biochemically derived biological products. Moreover quality control (QC) is of high importance due to potential manufacturing process variability and the living nature of the product. The development of therapeutic stem cell containing products such as 3D TE constructs, will require insightful QC that will guarantee identity, quality, purity, safety and potency. In most cases a QC pipeline will also help to further optimize the manufacturing processes. Cell identity is normally measured by marker gene and protein expression analysis using quantitative real time polymerase chain reaction (qPCR), enzyme-linked

immunosorbent assay (ELISA) and flow cytometry. For cells that satisfy this standard set of identity criteria, multilineage differentiation assays that are typically associated with stem cell properties will be needed to further identify stem cell populations. For example mesenchymal stem cells should be able to differentiate to the osteogenic, adipogenic, and chondrogenic lineage. However these established analysis methods are not the only cell-related QC options, non-invasive measurements could also provide information regarding the properties of the cultured cells and tissue. Upon thorough validation they could be sufficient for the determination of TE product quality attributes. For example such methods have been employed for the determination of cell content during perfusion bioreactor operation via metabolic activity assays (Zhou et al., 2013b) oxygen consumption (Santoro et al., 2012, Lambrechts et al., 2014) heat production due to cell growth (Santoro et al., 2011) and metabolic imaging (Ward et al., 2013). However in order to obtain more insightful and accurate information, where volume averaged sensor read outs might prove insufficient, fluorescent based imaging of oxygen sensitive fluorescent microbeads (Lambrechts et al., 2013) could fill in the gap.

A crucial aspect towards non-invasive QC for TE products would the development of novel methods or the incorporation of existing technologies (ELISA, mass spectrometry) in bioreactor setups that would allow online biochemical readouts (i.e., gene expression, protein secretion, cell surface antigen expression). It should be noted that when dealing with clinical delivery of TE products, quality properties regarding spatial properties should also be taken into account. For example extracellular matrix quantity and its distribution throughout the produced TE construct require a tailored characterization approach. Imaging techniques such as computed tomography could provide possibilities to obtain in a non-destructive way this information for TE constructs (Papantoniou et al., 2013). Overall the QC strategy followed will be determined by the manufacturing context that will be required for the clinical delivery of the TE product (Figure 6).

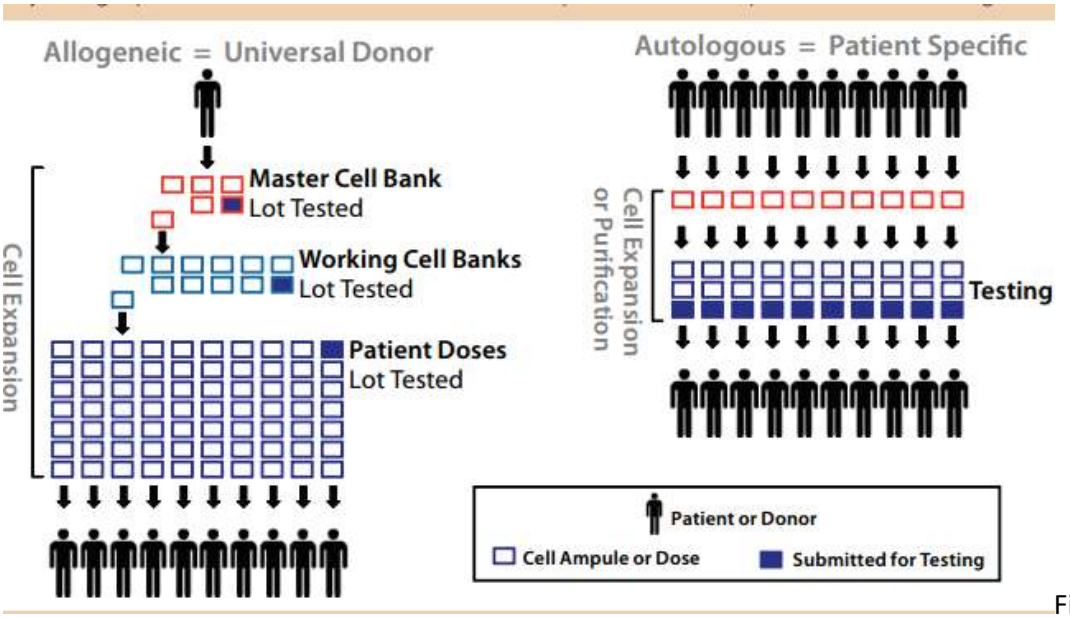

Figure 6: Quality control strategies for allogeneic and autologous TE products. For the first strategy representative lots could be tested due to the homogeneous starting cell population. In the second case a more stringent quality control strategy should be employed since the variability introduced in cell populations due to the variety of donors will impact on process performance and TE product quality attributes (Figure from Brandenberger R, Burger S, Campbell A, Fong T, Lapinskas E, Rowley JA.2011. Cell therapy Bioprocessing. BioProcess International, Vol. 9, No. S1, 2011, 30–37)

23.4 Online data-based monitoring- cross-talk between process parameters and TE construct quality attributes

On-line non-invasive bioprocess monitoring has gained attention since the FDA launched the process analytical technology guidelines (PAT initiative) in which biopharmaceutical companies are encouraged to adopt monitoring tools to ensure pre-defined final product quality (Teixeira et al., 2009). However, monitoring is only the first step towards the controlled production of tissue constructs with a robust clinical success rate. Ultimately an improved control of the TE construct quality is envisioned through the measuring, modeling and managing of critical cell culture parameters (M^3C) (Carrondo et al., 2012). The complexity and inherent variability of biological systems makes their control a challenging task. This becomes even more challenging when *in vitro* processes aim to mimic the developmental stages of embryo or organ maturation requiring spatial and temporal manipulation of the bioprocess (Lenas et al., 2009, Ingber et al., 2006). However interesting opportunities arise within GMP environments for TE production as large amounts of high-

quality data related to stem cell and TE construct properties will be continuously gathered. Examples are, among others, measurements on environmental culture conditions (dO2, pH, CO_2), image analysis (i.e. confluency, morphological features, collagen presence) and -omics datasets. With the rise of automated cell culture processes the amount of datasets will only increase, both sample-wise due to higher sampling frequency but also parameter-wise due to the development of new modalities. Monitoring of critical culture parameters on its own is key for process control and fault detection, but the real challenge will be to translate common process read-outs into interpretable TE construct quality attributes. At a final stage this could provide the potential to link end TE product characteristics to *in vivo* performance and ultimately, predict *in vivo* outcome upon implantation.

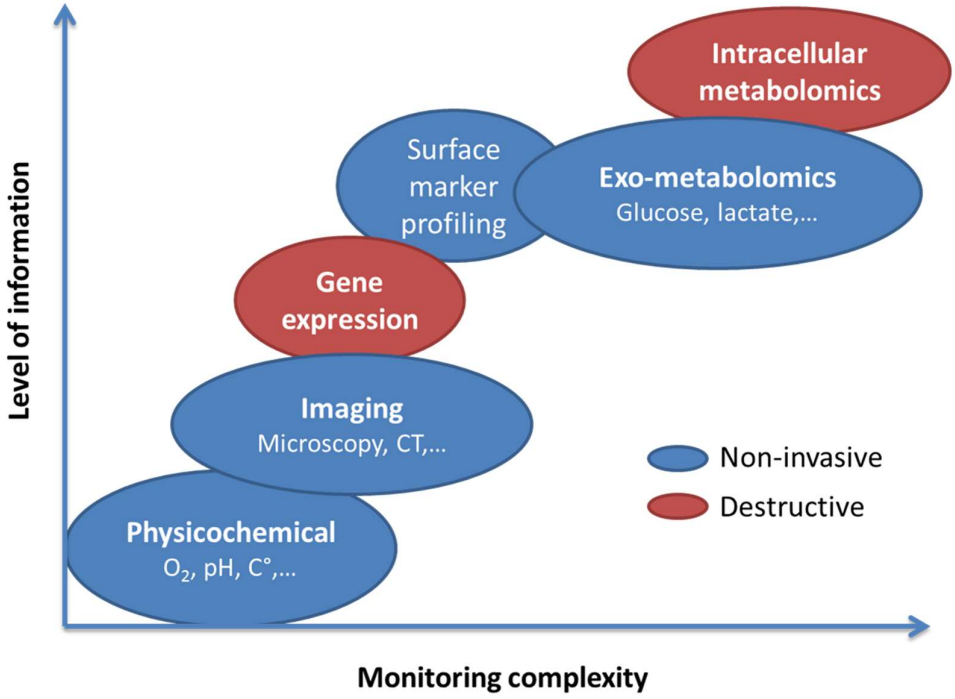

Figure 7: Schematic ranking of the trade-off between the complexity of analysis techniques and the level of information they can provide. The selection of the most appropriate analysis technique used is application specific. The challenge will be to gain the right level of information with (a combination of) the most accessible technologies.

23.4.1 Image data read outs

Visual inspection, either manual or automated, is common practice to assess cellular characteristics and can often be linked to for example stem cell differentiation. Certain

processes developments, such as the reprogramming success rate of iPS cells (Smith et al., 2010) or the likelihood of differentiation commitment (Roccio et al., 2013) can be non-destructively assessed in early culture phases with imaging based algorithms. The next step is to leverage this information from monitoring algorithms to predict quality attributes of the construct. For example Matsuoka et al. (Matsuoka et al., 2013) used phase-contrast microcopy to obtain time-series of morphological changes to subsequently develop a prediction model of osteogenic differentiation. In this model computational machine learning (ridge regression) was applied to make morphology-based predictions of cellular quality (ALP-activity and mineral deposition at day 14 and 21 respectively after osteogenic induction) taking into account patient specific variance (Figure 8). However this method is only suitable for 2D cell culture systems. The extra dimension when culturing in 3D TE constructs would require for more powerful imaging modalities such as computed tomography systems to be incorporated in bioreactor operation (Hagenmuller et al., 2010) hence obtaining spatial information regarding the whole tissue under development.

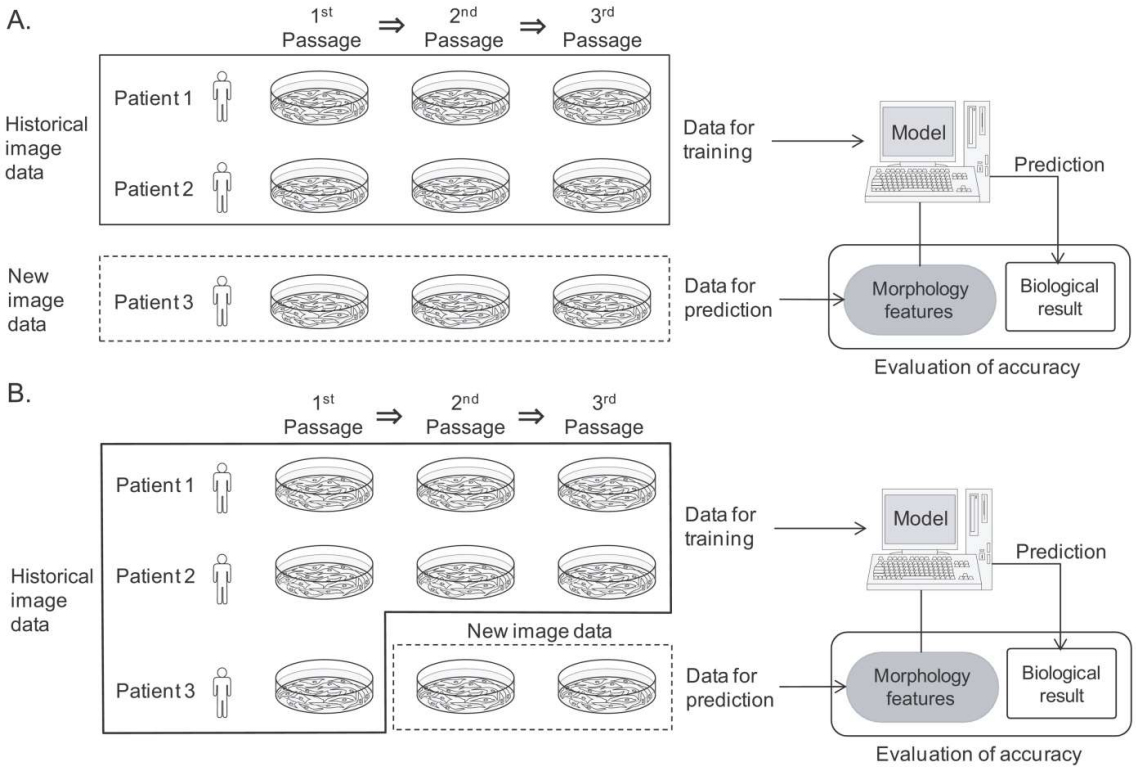

Figure 8: Historical image data collected from a range of patients were used to train algprithms (Scenario I). Images from all passages of patient 3 were used for prediction. B Ongoing prediction scheme: Trained by historical patient datasets and a partial dataset from

the new patient. For example for the prediction of cell potential of patient 3, Scheme I uses images patient 1 and 2 only. Scheme II uses images of patient 1 and 2, together with some images of patient 3. (Matsuoka et al., PLOS One, 2013. DOI: 10.1371/journal.pone.0055082)

23.4.2 Online sensor data read outs

The incorporation of multiple sensors to frequently monitor environmental culture conditions during culture has been suggested by PAT strategies. Although effective for process monitoring and fault detection, dO2 and pH read-outs are no direct measures of cell or tissue construct quality. Several strategies exist to indirectly extract more quantitative information on TE quality attributes such as cell number and cellular activity based on oxygen measurements in bioreactors (Santoro et al., 2012). Volkmer et al. (Volkmer et al., 2012) not only monitored the oxygen concentration within a TE scaffold, but also controlled the cellular microenvironment based on an oxygen concentration triggered feedback loop to steer the medium feed rate. However, provided the biological complexity of cells or a TE construct, it is unlikely that environmental physicochemical measurements alone will deliver a detailed signature of the construct quality and be able to robustly predict the clinical effectiveness of the construct. Meanwhile dynamics in metabolite accumulation or nutrient consumption have the potential to yield more in depth assessment of the cellular status (Patti et al., 2012). Glucose/lactate measurements (Schop et al., 2009), soluble signaling factors (Csaszar et al., 2013) and metabolic assays in bioreactors are currently being investigated (Zhou et al., 2013b). By measuring cellular metabolites that are secreted into the medium, so called exometabolomics, non-invasive information on the intracellular state up to the proteomic and genomic level can be obtained. Metabolomics and especially exometabolomics have great potential to become a non-invasive holistic physicochemical characterization of TE cultures.

23.4.3 Online data treatment

Ultimately the development of data-based algorithms that will be able to process data on line/ in real time, are essential for the automatic adjustment of bioprocess parameters based on variations of cell behavior (Carrondo et al., 2012). This evolution will be crucial for the final optimization of ATMP manufacturing protocols and for the delivery of reproducible high

quality ATMP end products. Such an approach, in synergy with already existing mechanistic approaches, will enhance our understanding of the highly complex TE bioprocesses and allow the development of predictive models for *in vitro* cell behavior, and hopefully *in vivo* outcome

In order to increase the TE construct quality, there is a need for mathematical approaches that are able to systematically integrate multiple data sources and provide us with predictive models for optimization and control of the TE processes. Generally, models can be divided in mechanistic models (deductive approach based on *a priori* knowledge) and data-driven models (inductive approach based on observed data). From the former, many examples can be found within the TE field under the form of conservation equations, stoichiometric models, reaction kinetics, etc. Data-based models are found within the biological process technology under the form of regression models, principal component analysis and neural networks. Mechanistic models may be superior in providing insight in the system under consideration however they require a significant cost of development, more difficult parameterization and are generally harder to solve. Data-based models provide less insight in the system, but in situations where the data logging surpasses the speed of analysis they provide an ideal basis for online prediction and control. An interesting direction regarding the GMP production of TE constructs, where both increased automation and process understanding are pursued, is the data-based mechanistic approach in which the initial "black-box" model identification stage is followed by a mechanistic interpretation of the model parameters and model structure (Table 3). This approach generally results in physically/biologically meaningful low order models that are able to describe the dominant behavior of a biological system and can be used in real-time controllers.

	Mechanistic models	Data-based models	Data-based mechanistic
Providing insight in system	+++	---	+
Cost of development	---	++	-
Online interrogation	--	++	++
Controller friendly	+	+++	+++

References	Aehle (2012), Kirouac (2009)	Roberts (2011), Volkmer (2012)	Lambrechts (2014), Ashoori (2009), Bennet (2008)

Table 3: Advantages and disadvantages of various modeling approaches.

23.5 Enhancing in vivo performance: an in silico mediated approach for TE product design

The challenge the TE field now faces is to translate (in vivo) regenerative processes to an in vitro environment on an engineering scale, thus making the process measurable and controllable with a limited number of parameters. Nature uses a very complex system of regulatory mechanisms compounded by a huge amount of redundancy to deal with the panoply of internal and external influences. The intricate interplay between all these factors is too complex to be interpreted without the help of computational, in silico, modeling (Jukes et al., 2008, Ingber et al., 2006).

23.5.1 Model variety and classification

In silico models exist in many shapes and forms, allowing for very different questions to be tackled. Typically, models are categorized based on the information they use to build the model (Janes et al., 2004). When using only experimental data (such as the large data sets generated by high throughput techniques), models are called empirical or data-driven. When they are based on researcher interpretation of the data, and the proposed hypotheses on the mechanisms of action, we talk about mechanistic or hypothesis-driven models. Another classification often used for in silico models is based on the spatio-temporal scales that are encompassed. Intracellular or subcellular models most often focus on the regulatory networks defining cellular behavior (Kinney and McDevitt, 2013). Cellular models consider cells as individual agents that can interact with each other and their environment. A level higher, the tissue/organ level, cells are represented in a more continuous way by means of their density. This level is typically suited to look at the overall effect of cellular actions and interactions in the form of neotissue formation in TE scaffolds, the influence of external influences such as mechanical modulators, nutrient supply and chemicals. Also the influence of the in vivo environment on the TE product is described at this level. Finally, at the population level, in silico clinical trials are increasingly carried out, especially in the field of critical care and cancer treatments (Roelofs et al., 2012), to optimize the trial design and

patient stratification as to limit the expenses and avoid negative side-effects as much as possible.

Given the current underlying difficulty in estimating parameter values, many models may remain limited in the mechanistic insight they provide and in their capability to predict system dynamics in unforeseen conditions. To investigate the impact of the chosen parameter values on the simulation results, sensitivity analyses should be carried out. Sensitivity analyses appear under many different forms, ranging from the most frequently used one-at-the-time (OAT) analysis where only one parameter is altered in each simulation to multifactorial DoE approaches run providing information on the interaction between experimental process parameters. Finally, in order to explicitly account for the noisy character of the data and processes, stochasticity can be introduced into the in silico models. Explicitly taking into account the uncertainty in the experimental data, can result in the retrieval of a wide range of parameter sets, all capable of fitting the data. Perhaps surprisingly, some predictions will still be very well constrained even in the face of this enormous parameter uncertainty, a property which was coined 'sloppy' (Gutenkunst et al., 2007). Even though sloppiness is not unique to biological systems, it is particularly relevant to biology because the collective behavior of most biological systems is much easier to measure in vivo than the values of individual parameters. Using sloppy parameter analysis (SPA), concrete predictions can be extracted from models long before their parameters are even roughly known (Brown et al., 2004), and when a system is not already well-understood, it can be more profitable to design experiments to directly improve predictions of interesting system behavior rather than to improve estimates of parameters. In order for TE to benefit maximally from the advantages in silico modeling can bring, an integrative approach is indispensable in which the development of the in vitro, in vivo and in silico components of research go hand in hand from the start.

23.5.2 In silico implantation of TE constructs

The host environment is a crucial component of the TE design strategy and the ability to predict the interaction between the host and the TE product is pivotal for many of the processes discussed in this chapter. In silico models allow to combine knowledge on basic biology and TE product behavior to study the effect of e.g. in vivo scaffold dissolution on

local in vivo cell biology (Carlier et al., 2011) and/or blood vessel formation (Checa and Prendergast, 2010). Patients presenting with structurally and/or genetically challenged healing environments pose additional challenges to the TE strategy but it is most often in those patients that normal healing is impaired and thus TE solutions are required. In silico models are applied to both study the etiology of impaired healing (Geris et al., 2010), and to design novel therapeutic strategies that are able to overcome the additional patient-specific hurdles. These models provide an additional level of (mechanistic) understanding to the data-driven empirical models which use multiparametric techniques to link in vitro characteristics to observed in vivo behavior. Taking into account that parameter quantification of scale-specific models is already difficult, the problem is even worse for multiscale models where parameters have to be defined at different scales. It is widely accepted that as more quantitative experimental data become available to build and constrain the parameter values, the models will be more likely to describe observed behaviors accurately.

23.5.3 Whole bioprocess virtual TE

One important application of mathematical models is the study of individual components of TE products such as the cells and the scaffold. Biological systems (TE product behavior being one of them) are naturally multiscale and to understand their behavior fully we must understand the interaction of a number of processes that may occur on diverse temporal and spatial scales. Models focusing on the cell compartment are used to identify the biological state of the cell based using statistical methods applied to large data sets (Mentink et al., 2013) or more mechanistic methods built on knowledge of specific relevant pathways. Combining both approaches allows identify the precise state of the cells of the TE product but also of optimized culture conditions providing the potential to push or keep cells in the desired state (see state of the art experiment, (Csaszar et al., 2012)). Models regarding the scaffold, focus on the mechanical (Song et al., 2013), biochemical (Demol et al., 2011, Bjork and Tranquillo, 2009) and morphological aspects (Saito et al., 2012) of its design with the aim of understanding its influence on the behavior of the seeded cells. More and more these in silico models are combined with a description of the physical environment that bioreactors presents to the TE product during the in vitro culture process (in terms of e.g. fluid flow, mechanical stimulation and mass transport)(Causin et al., 2013). Optimization of

initial cell seeding and initial cellular differentiation is predicted through the adaptation of the bioreactor protocol and scaffold morphology (Spencer et al., 2013). Additionally, neotissue growth during extended culture of the TE products can be captured by multiphysics models combining a description of the physical bioreactor environment with a mechanistic description of cellular behavior and matrix production (Guyot et al., 2014) (Figure 9). Once validated, these multi-physics models can become an inherent part of bioprocess development loop providing an insight view in the TE product in culture by allowing to liaise the bioreactor sensor read-outs (e.g. pressure drop) with the biological interpretation in terms of local neotissue growth in the TE product.

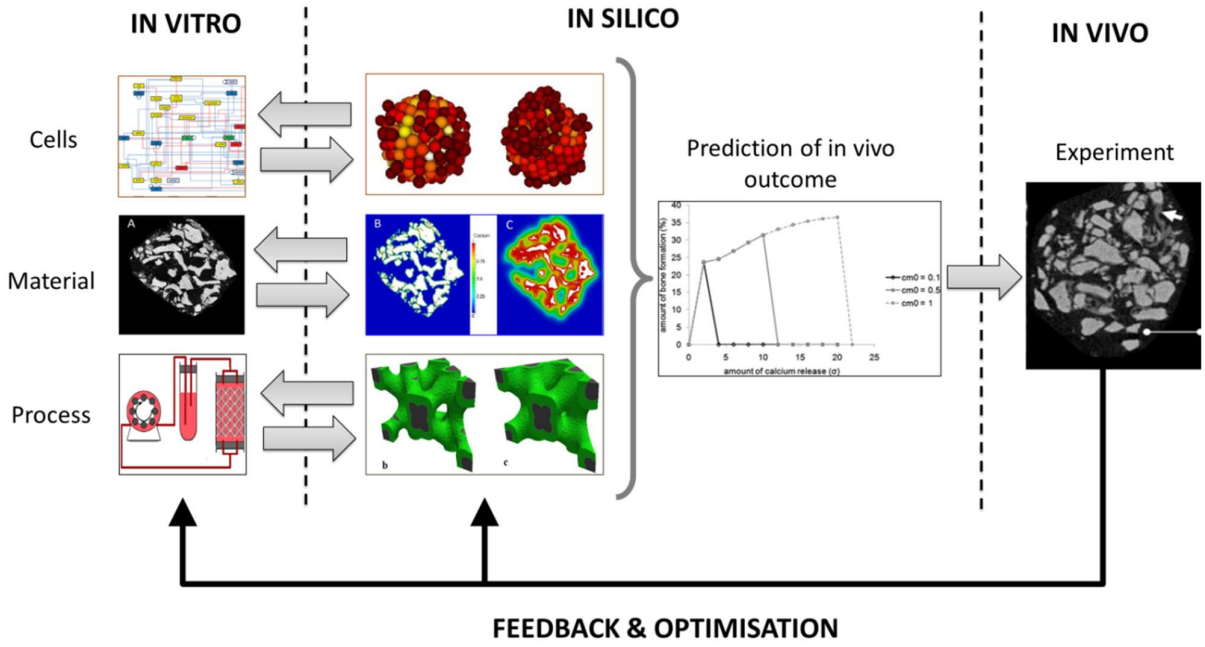

Figure 9: In vitro – in silico – in vivo optimization loop. The in vitro part (left) shows the raw materials of TE product manufacturing: cells (showing a gene regulatory network, Kerkhofs et al, PLoS ONE 2012), biomaterials (i.e. CaP-collagen carrier visualized with nano-Computed Tomography, courtesy G. Kerkchofs) and process environment (schematic representation of perfusion bioreactor set-up). The properties of these raw materials can be optimized using in silico models for all the constituents of a TE product (middle): cells (aggregating on a microbead, Smeets et al, CMBBE 2013), biomaterials (degradation & calcium release from calcium containing scaffolds, courtesy V. Manhas) and process environment (neotissue growth in scaffold, Guyot et al, BMMB 2014). Additionally, in silico models can combine the raw materials and make predictions on optimal combinations leading to maximal bone formation in vivo (influence of seeding density on bone formation in calcium containing scaffolds, Carlier et al, Acta Biomaterialia 2011). In vivo experiments (bone formation in CaP scaffolds, Roberts et al, Biomat 2012) close the in vitro-in silico-in vivo optimization loop and confirm the in silico predictions or suggest model adaptations and in more focused vitro experiments.

23.6 Downstream processing in TE manufacturing

As already discussed, automation of current lab scale processes for regenerative medicine and tissue engineering is widely considered to be essential for the successful clinical implementation and industrial translation of these emerging technologies (Ratcliffe et al., 2011, Abbasalizadeh and Baharvand, 2013). As already discussed in this chapter, the use of bioreactor systems is considered to be an indispensable step for cell culture and expansion as part of TE strategies. Although bioreactor systems already enable automated cell expansion in a GMP environment, a series of manual, operator dependent down-stream post processing steps are still required to deliver a finalized product. For example cell harvest steps for the recovery of cells from microcarriers or 3D cell aggregates and also subsequent seeding steps will be needed to reach the end product. Process steps such as cell harvest and up-concentration, can be managed on a lab scale for a limited amount of systems. However this lab scale approach poses severe limitations when scale-up and scale-out challenges need to be addressed, as it impacts on the quality of the manufactured TE products. Therefore, next to continuing to develop and optimize strategies enabling GMP compliant cell expansion, the development of suitable down-stream processing methods should receive equal attention.

23.6.1 Cell harvest/recovery

Cell harvest is proving to be one of the most challenging and critical process steps limiting the industrial implementation of current processes (Cierpka et al., 2013, Liu et al., 2013). Despite the development and intensive research activities in bioreactor technology development, subsequent processes are currently lacking equal attention. Enzymatic retrieval of the cells from the culture surface is the gold standard up till now despite the associated disadvantages such as (i) potential damage to the cells, (ii) cost of the procedure and (iii) availability of the GMP grade proteolytic dissociation enzymes (Abbasalizadeh and Baharvand, 2013, Cierpka et al., 2013). The long standing use of these reagents for cell recovery has however resulted in the development of highly efficient, minimally invasive methodologies which have already been successfully been implemented in commercial available systems (Roberts et al., 2012, Jones et al., 2013). Similar methodologies have been developed for micro-carrier based cell expansion (Chen et al., 2013a, Goh et al., 2013, Zhou et al., 2013a) although the versatility of this culture platform also enables to develop novel,

enzyme independent, cell recovery methods based on for example temperature dependent behavior of the surface (Tamura et al., 2012). Recently, the use of carrier-free culture methods for anchorage dependent cells based on cell aggregate formation has emerged as a novel, promising concept for cell culture. Although the classical enzymatic methods can be used to recover cells expanded in these culture systems (Abbasalizadeh et al., 2012a), the potential to develop enzyme-free methodologies has significant advantages such as the reduced consumable cost, reduced final volumes and the removal of a potential harmful reagent. Although enzyme-free methods for aggregate dissociation are currently being developed, they are often associated with a significant decrease in viability and other adverse effects ranging from spontaneous differentiation to apoptosis (Abbasalizadeh and Baharvand, 2013, Ratcliffe et al., 2011, Cierpka et al., 2013). Novel methods are however being developed which might enable enzyme free cell recovery from micro-carrier and aggregate culture systems (Wallman et al., 2011). However, for 2D based systems such as multiplate stacks and hollow fibre systems enzymatic cell recovery will probably remain the gold standard. It should be noted that enzymatic dissociation media will require further optimization both in composition and concentrations and a validation in a 3D context should be also carried out. Recently Nienow et al (2014) introduced a dynamic harvest methodology to retrieve expanded cells from microcarriers. However, it is evident that only minimal information is currently available and there is ample space for cell harvest process optimization research efforts (Chen et al., 2013b).

23.6.2 Volume reduction

Volume reduction and washing process requirements will be directly linked to the harvest volume, which will be a consequence of the bioreactor platform employed for cell culture and also the volumes used during the harvest process. Independent of the culture and harvest methodology used, the recovered cells will in most cases be suspended in large volumes of medium requiring subsequent up-concentration prior to further processing. Dependent on the final application of the process, different strategies might be of interest although mostly the physical differences between the cells and the fluid in which they are suspended are exploited. When the process targets cell expansion for allogeneic products a closed production pipeline capable of continuously processing large volumes of cells should be aimed at such as continuous centrifugation (used for the collection of blood plasma (Burd

and Schembri, 1993)) or aqueous two-phase separation systems (based on solubility differences (Gonzalez-Gonzalez and Rito-Palomares, 2013)). Other, more recent, developments for constant cell separation aim at the use of microfluidic based devices such as shown in Figure 10 although significant progress still needs to be made in up and out scaling prior to industrial implementation (Autebert et al., 2012, Gossett et al., 2010). For autologous products the use of disposable systems is a more viable approach as it allows the use of simple, single use separation methods. At current different clinical grade expansion systems such as the Quantum bioreactor (Terumo BCT, USA) and VueLife culture bags (CellGenix, USA) use batch centrifugation for cell concentration (Rojewski et al., 2013, Spanholtz et al., 2011). The scale out potential of this methodology is however limited as it still requires a significant amount of manual handlings - and the integration of alternative strategies such as cross flow filtration might be required for the successful industrial implementation.

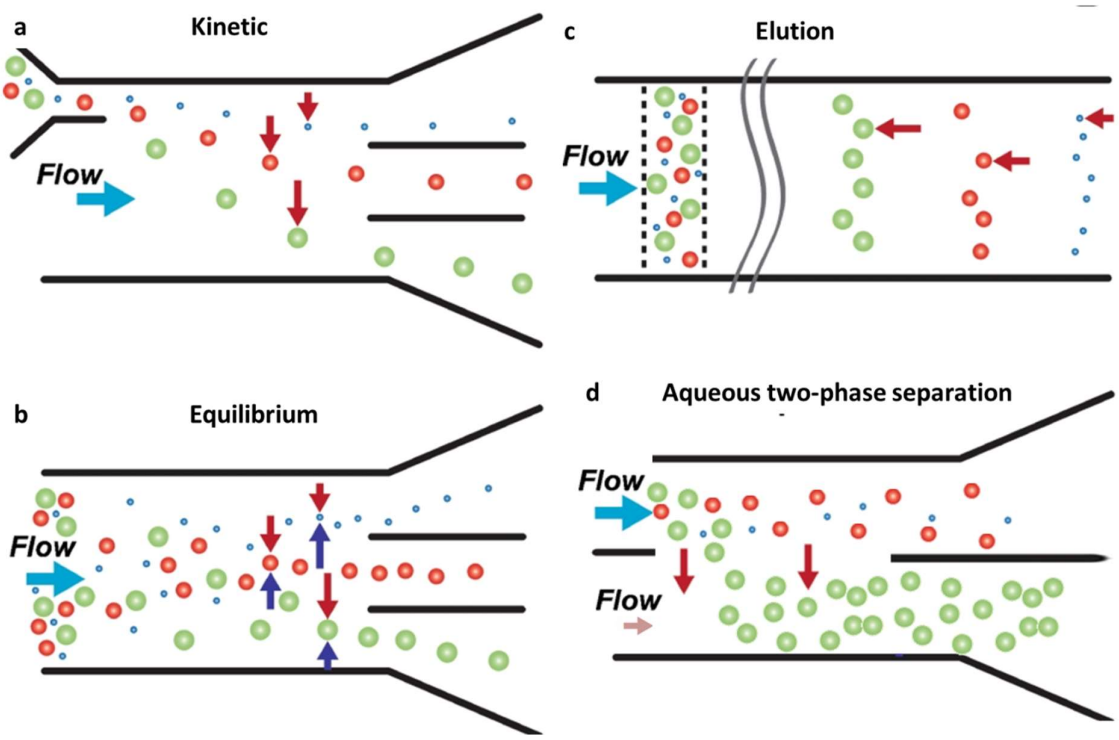

Figure 10: Generalized separation methods employed in microfluidic devices. a Continuous kinetic methods depend on the rate of cell deflection perpendicular to primary channel flow. b Continuous equilibrium methods involve migration to property-dependent equilibrium positions. c Elution methods depend on forces antiparallel to primary flow to create differential retention. d sub category of equilibrium based separation methods based on differences in solubility in two different aqueous phases. Figure adapted from (Gossett et al., 2010. Anal Bioanal Chem, 397:3249–3267).

23.6.3 Cell selection

Cell selection will predominantly be of importance before the cell expansion but the use of mixed cell populations could require additional purification steps subsequent to the harvest. Although a variety of methods is available ranging from separation based on physical properties to the use of aptamers and advanced tag free methods. Significant advances in process development are still required to enable their online implementation (Diogo et al., 2012). While separation based on physical properties such as centrifugation, aqueous two phase separation and filtration are reasonably high throughput these methods offer a low specificity, making them more suited for volume reduction than for high resolution cell selection (Abbasalizadeh and Baharvand, 2013, Diogo et al., 2012). Monoclonal antibody based techniques such as fluorescent activated cell sorting (FACS) and magnetic activated cell sorting (MACS) offer a significantly higher resolution although the labelling of cells with monoclonal antibodies might affect cell function and could induce unwanted responses (Abbasalizadeh and Baharvand, 2013). Aptamer based cell recognition is a more novel technique which might address these issues although obtaining a higher throughput becomes once more a limiting factor. The use of affinity chromatography based methods could enable the required high throughput and could also be integrated in both disposable systems for autologous therapy as well as in a production line for allogeneic products. In combination with additional recognition elements such as aptamers high specificity for the selection of a specific cell population could be obtained. The use of tag-free methodologies would however be more beneficial still. Parallelization of novel microfluidic based developments such as field flow fractionation and dielectrophoresis could enable high throughput separation of cell suspension, however these developments are still in their infancy and will require further development especially when scale up challenges will need to be faced (Abbasalizadeh and Baharvand, 2013, Fernandes et al., 2013, Diogo et al., 2012).

23.6.4 Integrating units of operation

Dependent on the culture system used, multiple challenges still need to be met to develop integrated, controlled and automated systems. Octane Biotech (Kingston, Canada) already delivered proof of concept with the development of the Cocoon system (Martin et al., 2009)

which allows the automated processing of a tissue biopsy into a tissue engineered construct using a closed and controlled environment. As this system was developed to facilitate the entire process from biopsy to final tissue engineered construct it includes all required up- and downstream processing modules. Once commercialized, it could offer great potential for bringing lab scale tissue engineering processes to the clinic. However in order to efficiently operate and optimize inter-dependable sequences of units of operation a 'whole-bioprocess' optimization strategy should be followed from early on in development. An understanding of how upstream processes will impact on the performance of subsequent process steps but also on final TE product quality attributes should be kept in mind during bioprocess design and development.

23.7 Towards efficient TE product translation

The term 'advanced' in the acronym ATMPs signifies the novelty and lack of prior history in dealing with living products. Hence the establishment of novel strategies that will have to emerge from a rapidly evolving research landscape is required. Major scientific breakthroughs, such as the introduction of iPS technology, can have major impact on the way 'current' manufacturing strategies may be structured up to that point. However the translation of scientific discoveries to clinic trials and subsequently to the market is a long procedure that will require input from various directions. Recent initiatives at both continental and global scale (European interdisciplinary summit on ATMPs, white paper REMEDIC, 2-3 May 2013, Vienna; Global assessment of stem cell engineering, 2014) have managed to conceptually streamline research developments in stem cell and tissue engineering into a more concrete direction towards translation to the clinic but also to the market. The joint 'forces' between academia industry and regulatory bodies will be key for the translation of TE products in a hospitable market/environment. Minimum requirements should be clearly defined and baselines given based on a constant discussion regarding international harmonization and standardization. Also some 'pioneer' products could set some initial guidelines towards this direction although enhanced communication and knowledge dissemination will be needed (See 23.1.2). Hospital exemption, minimizing national and subnational differences, reimbursement policies, predictive preclinical efficacy and safety testing, need for innovative systems for preclinical testing, product characterisation and product potency will have to be addressed. A recently published two

year global survey on stem cell engineering funded by the national science foundation (US), concluded that there needs to be an increased role for interdisciplinary research platforms where biologist and engineers and the engineering approach that will provide a foundation for the generation of new markets and future economic growth (Nerem et al, 2014. doi:10.1089/ten.tea.2013.0468). The recent establishment of institutes such as the Cell Therapy Catapult, London, UK (https://ct.catapult.org.uk/) and the Centre of commercialization of regenerative medicine Toronto Canada (http://www.ccrm.ca/), shows a trend of integration that is required for the realization of the hopes and promises of regenerative medicine.

There is need for technologies that will allow extract information from the performance and fate of implanted TE constructs in order to provide minimal quality requirements that should be met by the TE product quality profile. This will directly provide information for process engineers to target and customize accordingly the production pipeline to meet minimal criteria as obtained from in vivo monitoring. Regarding in vitro TE manufacturing platforms and practices, it may be stated that currently the production of sufficient quantities of stem cells (embryonic, iPS and mesenchymal) is feasible even in xenogeneic free platforms, as recently demonstrated by a number of publications. However further improvements in expansion and differentiation efficiencies based on the manipulation of process parameters are still achievable. Furthermore the incorporation of developmental biology principles during in vitro 3D TE production will require more elaborate strategies for efficient spatiotemporal delivery of the appropriate stimuli.

Future challenges in bioprocessing and manufacturing will include advanced and sophisticated monitoring platforms that allow monitoring at the cellular level. Developments in monitoring and control strategies that will be tailored for specific stem cell types but also for more complex 3D TE construct culture, should be pursued by the incorporation of multiple sensors in bioreactor systems. This would provide information- rich processes for the manufacturing of TE products that could meet regulatory demands. Furthermore monitoring robust bioprocesses (hence minimized variability) can provide high quality, low noise data for modelers in order to make more rapid progress in the years to come. Modeling approaches that will be able to capture the dynamic and transient nature of stem cell behavior by predicting characteristics not only of the final cell populations but also

of the 3D neotissue will be required. High throughput platforms will still be needed to determine optimal combinations between stem cell populations and biomaterial composition and texture but also for optimizing multicomponent differentiation media formulations.

Recently the development of decision tools that may screen and identify optimal manufacturing strategies, incorporating cost of goods (CoG) was demonstrated recently for an allogeneic therapy production scenario were various cell expansion strategies were compared computationally in terms of cost efficiency (Simaria et al., 2014) Figure 11). Similar studies should be include more units of operation taking into account risk assessment as introduced by donor to donor variability and its implications, especially for autologous production scenaria. The integration of bioprocess economics with bioprocess optimization to assess the economic competitiveness of available systems (i.e. planar, microcarrier-based cell expansion technologies) will be an asset to map optimum TE product translation strategies.

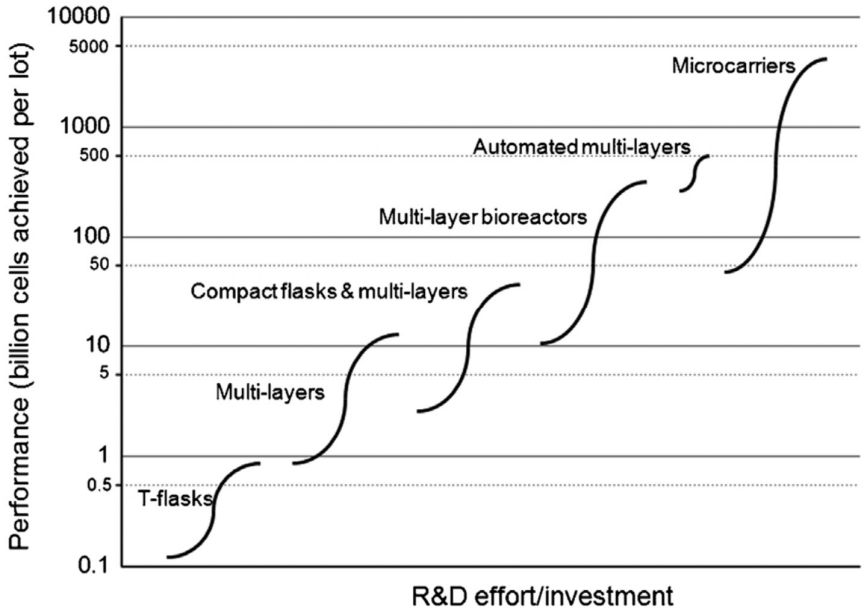

Figure 11: Conceptual illustration of a technology S-curve showing the evolution of expansion technologies used in cell therapy manufacture. The limits of each S-curve correspond to the amount of cells achieved by the smallest and largest size of each technology type when using the maximum number of units. Automated multi-layers refer to L-40 and cL-120. The x-axis represents qualitatively the R&D effort required for a company currently using T-flasks to change to other cell expansion technologies. (From Simaria et al., Allogeneic Cell Therapy Bioprocess Economics and Optimization: Single-Use Cell Expansion Technologies, Biotechnol. Bioeng., 111 (2014) 69-83).

State of the art experiment

Current expansion strategies, expose hematopoietic stem and progenitor cell (HSC) populations to uncontrolled culture environments with limited dynamics due to the unregulated accumulation of inhibitory endogenous factors. This results in compromises expansion levels that could ideally be obtained. In vitro (and in vivo) hematopoiesis is a dynamic and regulated process, through nonlinear feedback control. It should be noted that the rate of factor secretion varies widely among individual factors and does not always correlate with the exponential growth rates of total cell expansion. Overall a highly complex culture environment is developed with factor concentration dynamics following the dynamics of specific lineage subpopulations or resulting from multiple interacting feedback networks.

In a very interesting work, Csaszar et al. (2012) used a simplified model of HSC differentiation in which cell fates were regulated by feedback from secreted molecule cell interaction networks among various cells from different points in the developmental hierarchy. The model was employed to develop a culture strategy by which the controlled and specific inhibition of negative regulators of HSC differentiation allowed the global control of the cell population dynamics. As a preliminary step, they identified numerous factors present in cultures with the ability to inhibit the expansion of HSC and HSC

progenitor cells. In order to predict culture strategies that would maximize the abundance of HSC and progenitors, these factors were used as feedback candidates in the computational model. Using two commonly utilized bioreactor setups, namely fed-batch and perfusion systems, their calculations predicted that the fed-batch dilution approach would outperform other methods. When tested experimentally, the predicted protocol gave significantly higher average expansion of HSC and CD34+ progenitor cells after 12 days (Figure).

The ability to modulate secreted factor concentrations and measure corresponding functional outputs of cell expansions will allow for a more precise study of links between specific endogenous protein secretion and lineage subpopulations and their associated cell-cell interactions. This strategy serves as a robust clinically relevant system for rapid and automated in vitro cell expansion as well as a platform for further study of the regulation of cell-cell interactions in vitro and in vivo.

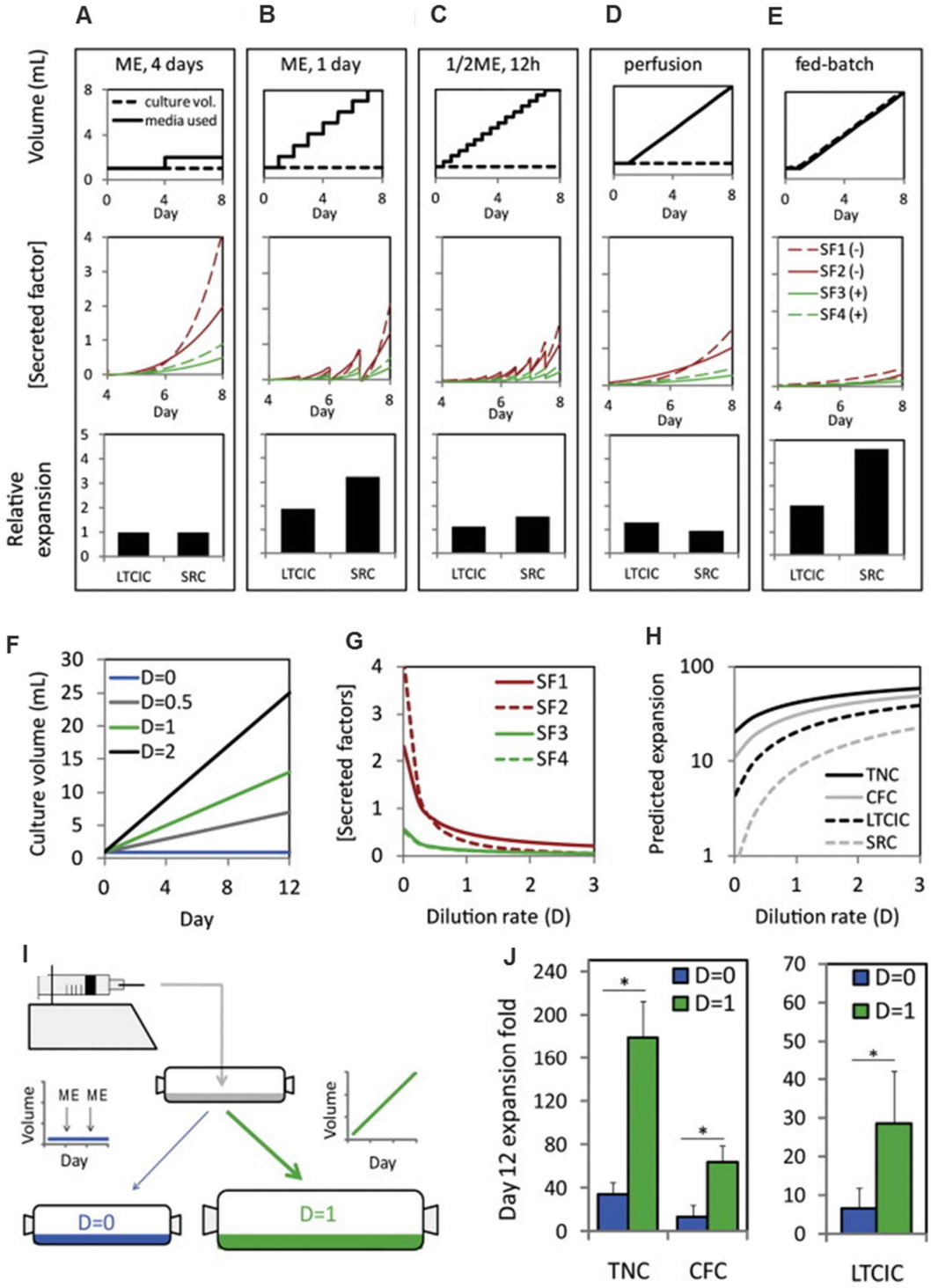

Figure: Computational simulations predict a Fed-batch strategy, at moderate dilution rates, to greatly reduce secreted factor concentrations enhancing cell expansion. (A–E) Simulated volume, secreted factor concentrations, and relative expansions under different media

manipulation strategies: (F) “control” culture with complete media exchange (ME) every 4 days; (G) culture with complete media exchange every 24 hr; (H) culture with 50% media exchange every 12 hr; (F) perfusion culture with one unit of media perfused every 24 hr; (G) fed-batch culture with one unit of media added every 24 hr. Conditions (F–H) are normalized to same media and cytokine requirements (one additional unit of media every 24 hr). (F) Media volume requirements for a fed-batch culture at different constant dilution rates, assuming a 1 ml initial volume. (G) Predicted effect of increasing constant dilution rate of fed-batch strategy on secreted factor concentrations. (H) Predicted effect of increasing constant dilution rate of fed-batch strategy on population expansions.
The Fed-batch strategy was experimentally validated in vitro to give significantly improved expansion of progenitor cells (I) Schematic of experimental set-up comparing control (D = 0) strategy with 100% media exchange (ME) every 4 days to fed-batch (D = 1) strategy. (J) Expansions of TNC, CFC, and LTC-IC, after 12 days of culture. n > 5. Total nucleated cells (TNCs), colony-forming cells (CFCs), long-term culture-initiating cells (LTC-ICs).

References

ABBASALIZADEH, S. & BAHARVAND, H. 2013. Technological progress and challenges towards cGMP manufacturing of human pluripotent stem cells based therapeutic products for allogeneic and autologous cell therapies. *Biotechnology Advances,* 31**,** 1600-1623.

ABBASALIZADEH, S., LARIJANI, M. R., SAMADIAN, A. & BAHARVAND, H. 2012a. Bioprocess development for mass production of size-controlled human pluripotent stem cell aggregates in stirred suspension bioreactor. *Tissue Eng Part C Methods,* 18**,** 831-51.

ABBASALIZADEH, S., LARIJANI, M. R., SAMADIAN, A. & BAHARVAND, H. 2012b. Bioprocess Development for Mass Production of Size-Controlled Human Pluripotent Stem Cell Aggregates in Stirred Suspension Bioreactor. *Tissue Engineering Part C-Methods,* 18**,** 831-851.

AUTEBERT, J., COUDERT, B., BIDARD, F. C., PIERGA, J. Y., DESCROIX, S., MALAQUIN, L. & VIOVY, J. L. 2012. Microfluidic: An innovative tool for efficient cell sorting. *Methods,* 57**,** 297-307.

BJORK, J. W. & TRANQUILLO, R. T. 2009. Transmural Flow Bioreactor for Vascular Tissue Engineering. *Biotechnology and Bioengineering,* 104**,** 1197-1206.

BROWN, K. S., HILL, C. C., CALERO, G. A., MYERS, C. R., LEE, K. H., SETHNA, J. P. & CERIONE, R. A. 2004. The statistical mechanics of complex signaling networks: nerve growth factor signaling. *Phys Biol,* 1**,** 184-95.

BURD, T. L. & SCHEMBRI, C. T. 1993. Separation chamber located radially outward from sample chamber and connected by flow restrictive channel. Google Patents.

CARLIER, A., CHAI, Y. C., MOESEN, M., THEYS, T., SCHROOTEN, J., VAN OOSTERWYCK, H. & GERIS, L. 2011. Designing optimal calcium phosphate scaffold-cell combinations using an integrative model-based approach. *Acta Biomater,* 7**,** 3573-85.

CARRONDO, M. J. T., ALVES, P. M., CARINHAS, N., GLASSEY, J., HESSE, F., MERTEN, O. W., MICHELETTI, M., NOLL, T., OLIVEIRA, R., REICHL, U., STABY, A., TEIXEIRA, A. P., WEICHERT, H. & MANDENIUS, C. F. 2012. How can measurement, monitoring, modeling and control advance cell culture in industrial biotechnology? *Biotechnology Journal,* 7**,** 1522-1529.

CAUSIN, P., SACCO, R. & VERRI, M. 2013. A multiscale approach in the computational modeling of the biophysical environment in artificial cartilage tissue regeneration. *Biomech Model Mechanobiol,* 12**,** 763-80.

CHECA, S. & PRENDERGAST, P. J. 2010. Effect of cell seeding and mechanical loading on vascularization and tissue formation inside a scaffold: A mechano-biological model using a lattice approach to simulate cell activity. *Journal of Biomechanics,* 43**,** 961-968.

CHEN, A. K., REUVENY, S. & OH, S. K. 2013a. Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: achievements and future direction. *Biotechnology Advances,* 31**,** 1032-46.

CHEN, A. K. L., REUVENY, S. & OH, S. K. W. 2013b. Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: Achievements and future direction. *Biotechnology Advances,* 31**,** 1032-1046.

CIERPKA, K., ELSEBERG, C. L., NISS, K., KASSEM, M., SALZIG, D. & CZERMAK, P. 2013. hMSC Production in Disposable Bioreactors with Regards to GMP and PAT. *Chemie Ingenieur Technik,* 85**,** 67-75.

CSASZAR, E., CHEN, K., CALDWELL, J., CHAN, W. & ZANDSTRA, P. W. 2013. Real-time monitoring and control of soluble signaling factors enables enhanced progenitor cell outputs from human cord blood stem cell cultures. *Biotechnol Bioeng*.

CSASZAR, E., KIROUAC, D. C., YU, M., WANG, W. J., QIAO, W. L., COOKE, M. P., BOITANO, A. E., ITO, C. & ZANDSTRA, P. W. 2012. Rapid Expansion of Human Hematopoietic Stem Cells by Automated Control of Inhibitory Feedback Signaling. *Cell Stem Cell,* 10**,** 218-229.

DEMOL, J., LAMBRECHTS, D., GERIS, L., SCHROOTEN, J. & VAN OOSTERWYCK, H. 2011. Towards a quantitative understanding of oxygen tension and cell density evolution in fibrin hydrogels. *Biomaterials,* 32**,** 107-18.

DIOGO, M. M., DA SILVA, C. L. & CABRAL, J. M. 2012. Separation technologies for stem cell bioprocessing. *Biotechnol Bioeng,* 109**,** 2699-709.

DOS SANTOS, F., ANDRADE, P. Z., ABECASIS, M. M., GIMBLE, J. M., CHASE, L. G., CAMPBELL, A. M., BOUCHER, S., VEMURI, M. C., DA SILVA, C. L. & CABRAL, J. M. S. 2011. Toward a Clinical-Grade Expansion of Mesenchymal Stem Cells from Human Sources: A Microcarrier-Based Culture System Under Xeno-Free Conditions. *Tissue Engineering Part C-Methods,* 17**,** 1201-1210.

DOS SANTOS, F. F., ANDRADE, P. Z., DA SILVA, C. L. & CABRAL, J. M. S. 2013. Bioreactor design for clinical-grade expansion of stem cells. *Biotechnology Journal,* 8**,** 644-654.

FAN, Y., HSIUNG, M., CHENG, C. & TZANAKAKIS, E. S. 2014. Facile engineering of xeno-free microcarriers for the scalable cultivation of human pluripotent stem cells in stirred suspension. *Tissue Eng Part A,* 20**,** 588-99.

FERNANDES, T. G., RODRIGUES, C. A. V., DIOGO, M. M. & CABRAL, J. M. S. 2013. Stem cell bioprocessing for regenerative medicine. *Journal of Chemical Technology and Biotechnology,* 89**,** 34-47.

FRENCH, A., BURE, K. & BRINDLEY, D. A. 2014. CASMI TSCC launch event, Paris, France, July 2013: an assessment of the key barriers to the commercialization and clinical adoption of pluripotent stem cell therapies. *Rejuvenation Res,* 17**,** 84-8.

FRITH, J. E., THOMSON, B. & GENEVER, P. G. 2010. Dynamic Three-Dimensional Culture Methods Enhance Mesenchymal Stem Cell Properties and Increase Therapeutic Potential. *Tissue Engineering Part C-Methods,* 16**,** 735-749.

FROHLICH, M., GRAYSON, W. L., MAROLT, D., GIMBLE, J. M., KREGAR-VELIKONJA, N. & VUNJAK-NOVAKOVIC, G. 2010. Bone grafts engineered from human adipose-derived stem cells in perfusion bioreactor culture. *Tissue Eng Part A,* 16**,** 179-89.

GERIS, L., REED, A. A. C., VANDER SLOTEN, J., SIMPSON, A. H. R. W. & VAN OOSTERWYCK, H. 2010. Occurrence and Treatment of Bone Atrophic Non-Unions Investigated by an Integrative Approach. *Plos Computational Biology,* 6.

GOH, T. K., ZHANG, Z. Y., CHEN, A. K., REUVENY, S., CHOOLANI, M., CHAN, J. K. & OH, S. K. 2013. Microcarrier culture for efficient expansion and osteogenic differentiation of human fetal mesenchymal stem cells. *Biores Open Access,* 2**,** 84-97.

GONZALEZ-GONZALEZ, M. & RITO-PALOMARES, M. 2013. Aqueous two-phase systems strategies to establish novel bioprocesses for stem cells recovery. *Critical Reviews in Biotechnology*.

GOSSETT, D. R., WEAVER, W. M., MACH, A. J., HUR, S. C., TSE, H. T. K., LEE, W., AMINI, H. & DI CARLO, D. 2010. Label-free cell separation and sorting in microfluidic systems. *Analytical and Bioanalytical Chemistry,* 397**,** 3249-3267.

GRAYSON, W. L., FROHLICH, M., YEAGER, K., BHUMIRATANA, S., CHAN, M. E., CANNIZZARO, C., WAN, L. Q., LIU, X. S., GUO, X. E. & VUNJAK-NOVAKOVIC, G. 2010. Engineering anatomically shaped human bone grafts. *Proc Natl Acad Sci U S A,* 107**,** 3299-304.

GUTENKUNST, R. N., WATERFALL, J. J., CASEY, F. P., BROWN, K. S., MYERS, C. R. & SETHNA, J. P. 2007. Universally sloppy parameter sensitivities in systems biology models. *Plos Computational Biology,* 3**,** 1871-78.

GUYOT, Y., PAPANTONIOU, I., CHAI, Y. C., VAN BAEL, S., SCHROOTEN, J. & GERIS, L. 2014. A computational model for cell/ECM growth on 3D surfaces using the level set method: a bone tissue engineering case study. *Biomech Model Mechanobiol*.

HAGENMULLER, H., HITZ, M., MERKLE, H. P., MEINEL, L. & MULLER, R. 2010. Design and validation of a novel bioreactor principle to combine online micro-computed tomography monitoring and mechanical loading in bone tissue engineering. *Rev Sci Instrum,* 81**,** 014303.

HUNT, M. M., MENG, G. L., RANCOURT, D. E., GATES, I. D. & KALLOS, M. S. 2014. Factorial Experimental Design for the Culture of Human Embryonic Stem Cells as Aggregates in Stirred Suspension Bioreactors Reveals the Potential for Interaction Effects Between Bioprocess Parameters. *Tissue Engineering Part C-Methods,* 20**,** 76-89.

INGBER, D. E., MOW, V. C., BUTLER, D., NIKLASON, L., HUARD, J., MAO, J., YANNAS, I., KAPLAN, D. & VUNJAK-NOVAKOVIC, G. 2006. Tissue engineering and developmental biology: Going biomimetic. *Tissue Engineering,* 12**,** 3265-3283.

JANES, K. A., KELLY, J. R., GAUDET, S., ALBECK, J. G., SORGER, P. K. & LAUFFENBURGER, D. A. 2004. Cue-signal-response analysis of TNF-induced apoptosis by partial least squares regression of dynamic multivariate data. *Journal of Computational Biology,* 11**,** 544-561.

JANSSEN, F. W., VAN DIJKHUIZEN-RADERSMA, R., VAN OORSCHOT, A., OOSTRA, J., DE BRUIJN, J. D. & VAN BLITTERSWIJK, C. A. 2010. Human tissue-engineered bone produced in clinically relevant amounts using a semi-automated perfusion bioreactor system: a preliminary study. *Journal of Tissue Engineering and Regenerative Medicine,* 4**,** 12-24.

JONES, M., VARELLA-GARCIA, M., SKOKAN, M., BRYCE, S., SCHOWINSKY, J., PETERS, R., VANG, B., BRECHEISEN, M., STARTZ, T., FRANK, N. & NANKERVIS, B. 2013. Genetic stability of bone marrow-derived human mesenchymal stromal cells in the Quantum System. *Cytotherapy,* 15**,** 1323-39.

JUKES, J. M., BOTH, S. K., LEUSINK, A., STERK, L. M. T., VAN BLITTERSWIJK, C. A. & DE BOER, J. 2008. Endochondral bone tissue engineering using embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 6840-6845.

KIM, J. & MA, T. 2012. Perfusion regulation of hMSC microenvironment and osteogenic differentiation in 3D scaffold. *Biotechnology and Bioengineering,* 109**,** 252-261.

KINNEY, M. A. & MCDEVITT, T. C. 2013. Emerging strategies for spatiotemporal control of stem cell fate and morphogenesis. *Trends Biotechnol,* 31**,** 78-84.

KIROUAC, D. C. & ZANDSTRA, P. W. 2008. The systematic production of cells for cell therapies. *Cell Stem Cell,* 3**,** 369-81.

LAMBRECHTS, D., ROEFFAERS, M., KERCKHOFS, G., ROBERTS, S. J., HOFKENS, J., VAN DE PUTTE, T., VAN OOSTERWYCK, H. & SCHROOTEN, J. 2013. Fluorescent oxygen sensitive microbead incorporation for measuring oxygen tension in cell aggregates. *Biomaterials,* 34**,** 922-9.

LAMBRECHTS, T., PAPANTONIOU, I., SONNAERT, M., SCHROOTEN, J. & AERTS, J. M. 2014. Model-based cell number quantification using online single-oxygen sensor data for tissue engineering perfusion bioreactors. *Biotechnol Bioeng*.

LENAS, P., MOOS, M. & LUYTEN, F. P. 2009. Developmental Engineering: A New Paradigm for the Design and Manufacturing of Cell-Based Products. Part I: From Three-Dimensional Cell Growth to Biomimetics of In Vivo Development. *Tissue Engineering Part B-Reviews,* 15**,** 381-394.

LIM, M., SHUNJIE, C., PANOSKALTSIS, N. & MANTALARIS, A. 2012. Systematic experimental design for bioprocess characterization: Elucidating transient effects of multi-cytokine contributions on erythroid differentiation. *Biotechnology and Bioprocess Engineering,* 17**,** 218-226.

LIU, N., ZANG, R., YANG, S. T. & LI, Y. 2013. Stem cell engineering in bioreactors for large-scale bioprocessing. *Engineering in Life Sciences,* 14**,** 4-15.

MAROLT, D., CAMPOS, I. M., BHUMIRATANA, S., KOREN, A., PETRIDIS, P., ZHANG, G., SPITALNIK, P. F., GRAYSON, W. L. & VUNJAK-NOVAKOVIC, G. 2012. Engineering bone tissue from human embryonic stem cells. *Proc Natl Acad Sci U S A,* 109**,** 8705-9.

MARTIN, I., SMITH, T. & WENDT, D. 2009. Bioreactor-based roadmap for the translation of tissue engineering strategies into clinical products. *Trends Biotechnol,* 27**,** 495-502.

MASON, C. & HOARE, M. 2007. Regenerative medicine bioprocessing: Building a conceptual framework based on early studies. *Tissue Engineering,* 13**,** 301-311.

MASON, C. & MANZOTTI, E. 2010. Regenerative medicine cell therapies: numbers of units manufactured and patients treated between 1988 and 2010. *Regenerative Medicine,* 5**,** 307-313.

MASON, C., MASON, J., CULME-SEYMOUR, E. J., BONFIGLIO, G. A. & REEVE, B. C. 2013. Cell Therapy Companies Make Strong Progress from October 2012 to March 2013 Amid Mixed Stock Market Sentiment. *Cell Stem Cell,* 12**,** 644-647.

MATSUOKA, F., TAKEUCHI, I., AGATA, H., KAGAMI, H., SHIONO, H., KIYOTA, Y., HONDA, H. & KATO, R. 2013. Morphology-Based Prediction of Osteogenic Differentiation Potential of Human Mesenchymal Stem Cells. *PLoS One,* 8.

MENTINK, A., HULSMAN, M., GROEN, N., LICHT, R., DECHERING, K. J., VAN DER STOK, J., ALVES, H. A., DHERT, W. J., VAN SOMEREN, E. P., REINDERS, M. J. T., VAN BLITTERSWIJK, C. A. & DE BOER, J. 2013. Predicting the therapeutic efficacy of MSC in bone tissue engineering using the molecular marker CADM1. *Biomaterials,* 34**,** 4592-4601.

NEREM, R. 2012. A global assessment of stem cell engineering. *Journal of Tissue Engineering and Regenerative Medicine,* 6**,** 406-406.

NOLD, P., BRENDEL, C., NEUBAUER, A., BEIN, G. & HACKSTEIN, H. 2013. Good manufacturing practice-compliant animal-free expansion of human bone marrow derived mesenchymal stroma cells in a closed hollow-fiber-based bioreactor. *Biochem Biophys Res Commun,* 430**,** 325-330.

PAPANTONIOU, I., CHAI, Y. C., LUYTEN, F. P. & SCHROOTEN, J. 2012. Process quality engineering for bioreactor-driven manufacturing of tissue engineered constructs for bone regeneration. *Tissue Eng Part C Methods*.

PAPANTONIOU, I., SONNAERT, M., GERIS, L., LUYTEN, F. P., SCHROOTEN, J. & KERCKHOFS, G. 2013. Three dimensional characterization of tissue-engineered constructs by contrast enhanced nanofocus computed tomography. *Tissue Eng Part C Methods*.

PATTI, G. J., YANES, O. & SIUZDAK, G. 2012. Metabolomics: the apogee of the omics trilogy. *Nature Reviews Molecular Cell Biology,* 13**,** 263-269.

PLACZEK, M. R., CHUNG, I. M., MACEDO, H. M., ISMAIL, S., BLANCO, T. M., LIM, M., CHA, J. M., FAUZI, I., KANG, Y., YEO, D. C. L., MA, C. Y. J., POLAK, J. M., PANOSKALTSIS, N. & MANTALARIS, A. 2009. Stem cell bioprocessing: fundamentals and principles. *Journal of the Royal Society Interface,* 6**,** 209-232.

RATCLIFFE, E., THOMAS, R. J. & WILLIAMS, D. J. 2011. Current understanding and challenges in bioprocessing of stem cell-based therapies for regenerative medicine. *British Medical Bulletin,* 100**,** 137-155.

RATHORE, A. S. & WINKLE, H. 2009. Quality by design for biopharmaceuticals. *Nature Biotechnology,* 27**,** 26-34.

ROBERTS, I., BAILA, S., RICE, R. B., JANSSENS, M. E., NGUYEN, K., MOENS, N., RUBAN, L., HERNANDEZ, D., COFFEY, P. & MASON, C. 2012. Scale-up of human embryonic stem cell culture using a hollow fibre bioreactor. *Biotechnology Letters,* 34**,** 2307-2315.

ROCCIO, M., SCHMITTER, D., KNOBLOCH, M., OKAWA, Y., SAGE, D. & LUTOLF, M. P. 2013. Predicting stem cell fate changes by differential cell cycle progression patterns. *Development,* 140**,** 459-470.

ROELOFS, E., ENGELSMAN, M., RASCH, C., PERSOON, L., QAMHIYEH, S., DE RUYSSCHER, D., VERHAEGEN, F., PIJLS-JOHANNESMA, M., LAMBIN, P. & CONSORTIUM, R. 2012. Results of a Multicentric In Silico Clinical Trial (ROCOCO) Comparing Radiotherapy with Photons and Protons for Non-small Cell Lung Cancer. *Journal of Thoracic Oncology,* 7**,** 165-176.

ROJEWSKI, M. T., FEKETE, N., BAILA, S., NGUYEN, K., FURST, D., ANTWILER, D., DAUSEND, J., KREJA, L., IGNATIUS, A., SENSEBE, L. & SCHREZENMEIER, H. 2013. GMP-compliant isolation and expansion of bone marrow-derived MSCs in the closed, automated device quantum cell expansion system. *Cell Transplant,* 22**,** 1981-2000.

SAITO, E., LIU, Y. F., MIGNECO, F. & HOLLISTER, S. J. 2012. Strut size and surface area effects on long-term in vivo degradation in computer designed poly(L-lactic acid) three-dimensional porous scaffolds. *Acta Biomater,* 8**,** 2568-2577.

SALTER, E., GOH, B., HUNG, B., HUTTON, D., GHONE, N. & GRAYSON, W. L. 2012. Bone tissue engineering bioreactors: a role in the clinic? *Tissue Eng Part B Rev,* 18**,** 62-75.

SANTORO, R., BRAISSANT, O., MULLER, B., WIRZ, D., DANIELS, A. U., MARTIN, I. & WENDT, D. 2011. Real-Time Measurements of Human Chondrocyte Heat Production During In Vitro Proliferation. *Biotechnology and Bioengineering,* 108**,** 3019-3024.

SANTORO, R., KRAUSE, C., MARTIN, I. & WENDT, D. 2012. On-line monitoring of oxygen as a non-destructive method to quantify cells in engineered 3D tissue constructs. *Journal of Tissue Engineering and Regenerative Medicine,* 6**,** 696-701.

SCHOP, D., JANSSEN, F. W., VAN RIJN, L. D. S., FERNANDES, H., BLOEM, R. M., DE BRUIJN, J. D. & VAN DIJKHUIZEN-RADERSMA, R. 2009. Growth, Metabolism, and Growth Inhibitors of Mesenchymal Stem Cells. *Tissue Engineering Part A,* 15**,** 1877-1886.

SCOTTI, C., PICCININI, E., TAKIZAWA, H., TODOROV, A., BOURGINE, P., PAPADIMITROPOULOS, A., BARBERO, A., MANZ, M. G. & MARTIN, I. 2013. Engineering of a functional bone organ through endochondral ossification. *Proceedings of the National Academy of Sciences of the United States of America,* 110**,** 3997-4002.

SIMARIA, A. S., HASSAN, S., VARADARAJU, H., ROWLEY, J., WARREN, K., VANEK, P. & FARID, S. S. 2014. Allogeneic Cell Therapy Bioprocess Economics and Optimization: Single-Use Cell Expansion Technologies. *Biotechnology and Bioengineering,* 111**,** 69-83.

SMITH, Z. D., NACHMAN, I., REGEV, A. & MEISSNER, A. 2010. Dynamic single-cell imaging of direct reprogramming reveals an early specifying event. *Nature Biotechnology,* 28**,** 521-U188.

SONG, M. J., DEAN, D. & TATE, M. L. K. 2013. Mechanical modulation of nascent stem cell lineage commitment in tissue engineering scaffolds. *Biomaterials,* 34**,** 5766-5775.

SPANHOLTZ, J., PREIJERS, F., TORDOIR, M., TRILSBEEK, C., PAARDEKOOPER, J., DE WITTE, T., SCHAAP, N. & DOLSTRA, H. 2011. Clinical-Grade Generation of Active NK Cells from Cord Blood Hematopoietic Progenitor Cells for Immunotherapy Using a Closed-System Culture Process. *PLoS One,* 6.

SPENCER, T. J., HIDALGO-BASTIDA, L. A., CARTMELL, S. H., HALLIDAY, I. & CARE, C. M. 2013. In silico multi-scale model of transport and dynamic seeding in a bone tissue engineering perfusion bioreactor. *Biotechnology and Bioengineering,* 110**,** 1221-1230.

TAMURA, A., KOBAYASHI, J., YAMATO, M. & OKANO, T. 2012. Temperature-responsive poly(N-isopropylacrylamide)-grafted microcarriers for large-scale non-invasive harvest of anchorage-dependent cells. *Biomaterials,* 33**,** 3803-12.

TEIXEIRA, A. P., OLIVEIRA, R., ALVES, P. M. & CARRONDO, M. J. T. 2009. Advances in on-line monitoring and control of mammalian cell cultures: Supporting the PAT initiative. *Biotechnology Advances,* 27**,** 726-732.

TIMMINS, N. E., KIEL, M., GUNTHER, M., HEAZLEWOOD, C., DORAN, M. R., BROOKE, G. & ATKINSON, K. 2012. Closed system isolation and scalable expansion of human placental mesenchymal stem cells. *Biotechnology and Bioengineering,* 109**,** 1817-1826.

VOLKMER, E., OTTO, S., POLZER, H., SALLER, M., TRAPPENDREHER, D., ZAGAR, D., HAMISCH, S., ZIEGLER, G., WILHELMI, A., MUTSCHLER, W. & SCHIEKER, M. 2012. Overcoming hypoxia in 3D culture systems for tissue engineering of bone in vitro using an automated, oxygen-triggered feedback loop. *Journal of Materials Science-Materials in Medicine,* 23**,** 2793-2801.

WALLMAN, L., AKESSON, E., CERIC, D., ANDERSSON, P. H., DAY, K., HOVATTA, O., FALCI, S., LAURELL, T. & SUNDSTROM, E. 2011. Biogrid--a microfluidic device for large-scale enzyme-free dissociation of stem cell aggregates. *Lab Chip,* 11**,** 3241-8.

WANG, Y., CHENG, L. & GERECHT, S. 2013a. Efficient and Scalable Expansion of Human Pluripotent Stem Cells Under Clinically Compliant Settings: A View in 2013. *Ann Biomed Eng*.

WANG, Y., CHOU, B. K., DOWEY, S., HE, C. X., GERECHT, S. & CHENG, L. Z. 2013b. Scalable expansion of human induced pluripotent stem cells in the defined xeno-free E8 medium under adherent and suspension culture conditions. *Stem Cell Res,* 11**,** 1103-1116.

WARD, A., QUINN, K. P., BELLAS, E., GEORGAKOUDI, I. & KAPLAN, D. L. 2013. Noninvasive Metabolic Imaging of Engineered 3D Human Adipose Tissue in a Perfusion Bioreactor. *PLoS One,* 8.

WENDT, D., RIBOLDI, S. A., CIOFFI, M. & MARTIN, I. 2009. Bioreactors in tissue engineering: scientific challenges and clinical perspectives. *Adv Biochem Eng Biotechnol,* 112**,** 1-27.

YEATTS, A. B., BOTH, S. K., YANG, W., ALGHAMDI, H. S., YANG, F., FISHER, J. P. & JANSEN, J. A. 2014. In vivo bone regeneration using tubular perfusion system bioreactor cultured nanofibrous scaffolds. *Tissue Eng Part A,* 20**,** 139-46.

YEATTS, A. B. & FISHER, J. P. 2011. Bone tissue engineering bioreactors: Dynamic culture and the influence of shear stress. *Bone,* 48**,** 171-181.

YEO, D., KIPARISSIDES, A., CHA, J. M., AGUILAR-GALLARDO, C., POLAK, J. M., TSIRIDIS, E., PISTIKOPOULOS, E. N. & MANTALARIS, A. 2013. Improving Embryonic Stem Cell Expansion through the Combination of Perfusion and Bioprocess Model Design. *PLoS One,* 8.

ZHOU, L., KONG, J., ZHUANG, Y., CHU, J., ZHANG, S. & GUO, M. 2013a. Ex vivo expansion of bone marrow mesenchymal stem cells using microcarrier beads in a stirred bioreactor. *Biotechnology and Biopocess Engineering,* 18**,** 173-184.

ZHOU, X. H., HOLSBEEKS, I., IMPENS, S., SONNAERT, M., BLOEMEN, V., LUYTEN, F. & SCHROOTEN, J. 2013b. Noninvasive Real-Time Monitoring by AlamarBlue (R) During In Vitro Culture of Three-Dimensional Tissue-Engineered Bone Constructs. *Tissue Engineering Part C-Methods,* 19**,** 720-729.

ZWEIGERDT, R., OLMER, R., SINGH, H., HAVERICH, A. & MARTIN, U. 2011. Scalable expansion of human pluripotent stem cells in suspension culture. *Nature Protocols,* 6**,** 689-700.

Dear authors,
Thank you very much for submitting the chapter on quality control, it is a very useful chapter in the textbook, marking the maturation of our field to one where the products get more and more attention. There are a few points of attention:
The text is rather abstract and generalistic. I think it is didactically beneficial you can weave in some practical examples.
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Best wishes,
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