and subchondral bone remodelling. While OA is associated with a huge societal and economic burden, no efficient disease-modifying drug still exists today. In addition to obesity, metabolic disorders, and trauma, one of the major risks of OA is ageing. Ageing is a complex physiological process that relates to a gradual decline in certain functions, coordination, loss of homeostasis, and physiological integrity. Among the underlying mechanisms associated with age-related diseases including OA, an accumulation of senescent cells has been observed. Interestingly enough, the elimination of such senescent cells has been shown to reduce pain and increase cartilage regeneration in murine post-traumatic OA. To further decipher the role of senescence in OA onset and development, our work aims to describe the location of senescent cells *in vivo* within the different joint tissues involved in age-related OA as well as investigate the *in vitro* senescence-associated inflammatory response of chondrocytes.

Methods: To assess the expression of senescence markers during age-related OA, knee joints of male C57BL/6 mice of increasing age (1, 6, 12, 18, and 24 months) were harvested. Mice knee joints (n=5) of each age group were decalcified and embedded in paraffin. 5μm sections were stained with Safranin-O for OARSI scoring. Immunohistochemistry for senescence markers ($p21^{CIP-1}$, $p16^{INK4A}$, PAI-1, and caveolin-1 (CAV-1)), as well as cartilage extracellular matrix proteins (type II collagen, and the aggrecan neoepitope NITEGE), were performed. *In vitro*, to investigate the relationship between inflammation and senescence in chondrocytes, TC28a2 chondrocyte cell line and primary human articular chondrocytes (hAC) seeded at 15,000 cells /cm^2 were stimulated with IL-1β (10 ng/ml; 24 to 72h). To induce senescence, TC28a2 and hAC were seeded at 15,000 cells /cm^2 and treated with etoposide (20μM; 24h) and maintained in culture up to 192h. The effects of IL-1β and etoposide on senescence ($p16^{Ink4A}$, $p21^{CIP1}$, p53, PAI-1) and chondrocyte markers (MMP3, MMP13, COL2A1, and ACAN) were evaluated at the transcript and protein levels.

Results: Our *in vivo* results showed a gradual increase in the expression of senescence markers ($P16^{INK4A}$, $P21^{CIP-1}$, PAI-1, and CAV-1) with increasing ages, which correlates with an increase in OA score as well as the detection of the aggrecan degradation neoepitope NITEGE. Consistently, an age-related decreased in type II collagen was observed in the knee joints of mice with escalating ages. IL 1-β treatment of TC28a2 and hAC resulted in a decrease in COL2A1 and ACAN at 48h and 72h. As expected, an increase in inflammatory (COX2) and catabolic markers (MMP-3 and MMP-13) from 24h to 72h, was observed. However, no significant modification of senescence markers ($p16^{Ink4A}$, $p21^{CIP1}$, p53, and PAI-1) expressions was observed. Etoposide treatment induced a drop in cell proliferation from 24h to 72h before a stabilization in the numbers of viable cells up to 192h in both cell types. Following etoposide treatment, we observed a significant decrease in COL2A1 expression at 72h in both cell types and the induction of MMP-3 and MMP-13 at 24h and 72h in human chondrocytes. Etoposide treatment also triggered an augmentation of the senescence markers $p21^{CIP1}$ and PAI-1 as early as 24h in both types of chondrocytes.

Conclusions: To summarize, our *in vivo* Results highlights that senescence increases in correlation with age and OA severity in a spontaneous age-related OA murine model. Our *in vitro* data also suggest that IL-1β, a cytokine commonly used to *in vitro* simulate the OA-associated inflammation, did not induce a marked cellular senescence in human chondrocytes when cultured in our conditions. On the contrary, the topoisomerase inhibitor, etoposide was found to induce senescence in the TC28a2 chondrocytic cell line and primary human articular chondrocytes. This model of etoposide-induced senescence in chondrocytes could be a useful tool to study the complex role of senescence in OA chondrocytes as well as evaluate anti-OA therapeutic approaches targeting senescence.

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PRESENTATION NUMBER: 124
DEVELOPMENT OF A PRIMARY SKELETAL MUSCLE CELL CULTURE MODEL FROM HUMAN MUSCLE BIOPSIES

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Purpose: This work aimed to develop and validate a culture model of primary human skeletal muscle cells.

Methods: Muscle biopsies were obtained post-mortem from the *vastus lateralis*. The biopsies were enzymatically digested with collagenase 1A (1mg/mL) and dispase II (4,8mg/mL) for 60 minutes at 37°C in order to isolate fibroblasts and satellite cells. Then, the satellite cells (CD56+) were separated from fibroblasts (CD56-) by two methods: pre-plating or immunomagnetic cell sorting using an anti-CD56 antibody, a transmembrane glycoprotein expressed on the surface of satellite cells. Indeed, some human muscle biopsies contain a high proportion of fibroblasts that can contaminate the myoblasts cell culture if the separation method is not efficient. The performance of these cell separation methods was evaluated by FACS. We also compared the effects of two culture media on myoblasts proliferation (10%FBS vs. 10%FBS + 2% Ultroser G (UG)) using cell doubling time as parameter. Two differentiation media (2%FBS vs. 0.5%UG) were tested on the myogenic index, which corresponds to the proportion of cells that have fused to form myotubes. This index was determined through the detection of the myosin heavy chain (MYH) by immunofluorescence. Finally, we studied the expression of several muscle cell markers during myogenesis by qRT-PCR and immunofluorescence.

Results: The cells obtained from the enzymatic digestion of the biopsy contained a varying proportion of CD56+ satellite cells (86,15 ± 12,09%; 95%CI:73,45 to 98,84). The cell population obtained after immunomagnetic cell sorting method contained more than 92% of CD56+ cells (96,11 ± 3,27%; 95%CI:92,68 to 99,54). In comparison, the ratio of CD56+ obtained by the preplating method was lower (84,19 ± 9,94%; 95% CI:73,76 to 94,62), but no significant difference was observed between both methods. The cell doubling time was significantly shorter with culture media containing 10%FBS and 2%UG than with the media containing only 10%FBS (25.70 ± 3,96h vs. 70.64 ± 21,68h, $p = 0.0313$). The myogenic index was slightly higher with the 0.5%UG than 2%FBS differentiating medium (62,75% ± 8,57% vs. 56,39 ± 16,45%). Finally, the increase of MyoD1, myogenin and MYH, three markers of differentiation, indicated the phenotypic transition of myoblasts to a myogenic phenotype.

Conclusions: Immunomagnetic cell sorting allows better satellite cells and fibroblasts separation than the conventionally used preplating technique. Ultoser G serum substitute accelerates cell proliferation and improves the differentiation of myoblasts into myotubes. The markers MyoD1, myogenin and MYH are good markers of the myogenic differentiation of myoblasts. This *in vitro* model makes it possible to purify efficiently muscle satellite cells from human muscle biopsies. These cells can be used in *in vitro* studies to study the pathophysiological mechanisms of muscle disease and the mechanisms of action of treatments.

PRESENTATION NUMBER: 125
DEVELOPMENT OF A HUMAN OSTEOCHONDRAL CONSTRUCT ON A MICROFLUIDIC CHIP – TO ADVANCE FUNCTIONAL STUDIES OF OSTEOARTHRITIS RISK GENES

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Purpose: To advance functional follow up research of strong OA risk genes that have been frequently found to play a role in the osteochondral unit of the joint tissues, we developed a human *in-vitro* model system, mimicking the interacting joint tissues bone and cartilage.

Methods: The microfluidic chip used in this study consists of a chondrogenic and an osteogenic channel, separated by an electrospun polycaprolactone (PCL) matrix (Fig. 1). Primary osteogenic cells were seeded in the PCL matrix. After culturing the osteogenic cells for seven days, primary chondrocytes were seeded in the chondrogenic compartment, on top of the PCL matrix (RAAK-study, N=8 chips, N=4 patients). After a total culture period of 28 days, the chips were sacrificed and matrix production and gene expression were analyzed by histology and RT-qPCR. As a reference, we included 3D cell pellet