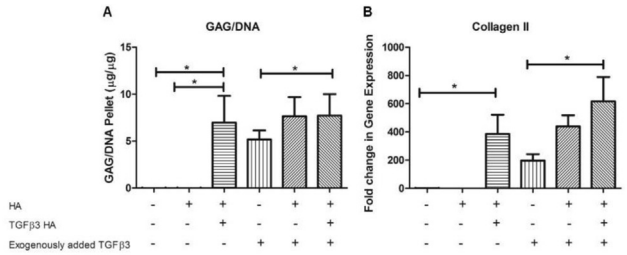


**Figure 1:** *In vitro* chondrogenesis of hMSCs. Results indicate a significant increase in GAG deposition and collagen type II expression with the addition of TGFβ3 HA microspheres. $^{*}p = \leq 0.05$

**Purpose:** Transforming growth factor beta (TGF-β) is an important regulator in determining cell fate of human mesenchymal stem cells (hMSCs), specifically down the chondrogenic lineage. Yet control of this differentiation within a complex in vivo milieu remains a challenge. A localized therapy such as an intra-articular injection of a system for controlled release of TGF-β3 would represent a readily translatable approach for in situ differentiation and remove the need for continuous supplementation of growth factors. Hyaluronan (HA) is an essential component of cartilage extracellular matrix (ECM) and serves as a support for cell adhesion, spreading and subsequently, cell growth. Thus, our aim was to fabricate chondromimetic HA microspheres and test these as extracellular growth factor targeting depots for initiation of in vitro and ex vivo chondrogenesis of hMSCs.
**Methods:** HA microspheres were fabricated using an electrostatic layer-by-layer strategy and characterized by scanning electron microscopy. hMSCs isolated from the iliac crest of healthy donors were obtained with approval from the National University of Ireland Galway and associated University College Hospital ethics committees and cultured in α-MEM containing 10% FBS, 100U/ml penicillin and 100µg/ml streptomycin. The effects of microspheres on cell number and metabolic activity were determined using the PicoGreen dsDNA® and alamarBlue™ assays. Confocal microscopy, flow cytometry and transmission electron microscopy were utilized to investigate co-localization of HA microspheres with hMSCs to ensure they were not taken up by cells. TGF-β3 was loaded onto HA microspheres via passive diffusion (400ng/mg), incubated with hMSCs and cultured for 21 d in incomplete chondrogenic medium (ICM) or complete chondrogenic medium (CCM) (+10ng/ml TGF-β3 every 2 d) to assess in vitro chondrogenesis. Glycosaminoglycan (GAG) measurement (DMMB assay), chondrogenic transcripts (qRT-PCR) and immunohistochemical staining (IHC) were used to validate successful chondrogenesis. For ex vivo culture experiments, fresh human articular cartilage samples were obtained under institutionally approved protocols from Merlin Park Hospital, Galway from donors who had undergone knee replacement surgery. Cartilage explants were allowed to equilibrate at 37°C for 48 h in Dulbecco's Modified Eagle's Medium (DMEM) + 10% fetal bovine serum and subsequently equilibrated for 24 h in ICM in an agarose well prior to switching to ICM or CCM media and treated with or without TGF-β3 loaded microspheres for 21 d culture. Experiments were performed for 3 donors with each experiment containing a minimum of three biological replicates. Statistical significance was assessed using one-way or two-way ANOVA followed by Tukey or Bonferroni post hoc analysis and denoted significant for $p = \leq 0.05$.
**Results:** HA microspheres retained their spherical morphology with uniform size distribution. Cellular compatibility studies indicated no adverse effects of 10µm HA microspheres. Following TGF-β3 loading of microspheres, incubation with hMSCs for 21 d in vitro pellet culture revealed enhanced accumulation of GAGs (Fig. 1A) and positive IHC of collagen type II in hMSCs incubated with growth factor loaded HA microspheres when compared to control cells. Additionally, qRT-PCR analysis from 14 d pellets revealed enhanced expression of aggrecan and collagen type II (Fig. 1B). 21 d explant culture indicated localization of TGF-β3 HA microspheres within fibrillations of osteoarthritic cartilage with implanted hMSCs and local chondrocytes showing increased GAG deposition compared to hMSCs alone (Fig. 2, arrow indicative of increased GAG staining in surrounding cells).
**Conclusion:** Injectable biomaterials hold great promise for orthopaedic applications, in particular for repair of articular cartilage. Preliminary in vitro/ex-vivo evidence presented here supports the use of TGF-β3-loaded HA microspheres for in situ differentiation of hMSCs.

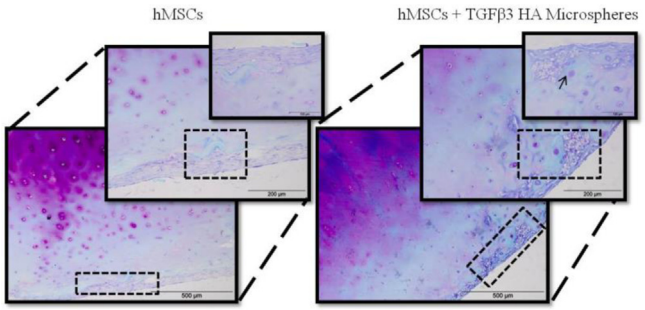


**Figure 2:** Ex-vivo culture of human articular cartilage explants for 21 d in ICM with human mesenchymal stems with or without TGFβ3 loaded HA microspheres.

## 886
## PRODUCING ARTIFICIAL CHONDRONS FOR IMPROVED CARTILAGE REPAIR

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**Purpose:** Articular cartilage has a limited capacity for self-repair and untreated damage often leads to the development of osteoarthritis and eventually joint failure. Currently, autologous chondrocyte implantation is the most successful method for repairing focal cartilage defects. This therapy involves the isolation of chondrocytes from articular cartilage by enzymatic degradation. This not only strips the cells from their extra cellular matrix, but also from their highly specialized microenvironments, called chondrons. This initiates dedifferentiation, i.e. the isolated chondrocytes progressively lose their chondrogenic phenotype, and thus reduces the clinical success of the therapy. We aim to develop a strategy to encapsulate expanded single chondrocytes in artificial chondrons. When successful, this approach represents a cost-effective method to prevent further dedifferentiation, while simultaneously allows for optimal stimulation of the encapsulated chondrocytes. Here, we present a microfluidic device able to encapsulate single chondrocytes in chondron-sized (<50 µm) microgels in a custom designable artificial matrix using an efficient and cell-friendly manner.
**Methods:** The master mould for the optimized microfluidic droplet generator was made by micropatterning ~25 µm thick SU8-50 (MicroChem) on a silicon wafer using standard photolithography techniques. PDMS (Sylgard 184, Dow Corning) was thermally cured on the master and bonded to glass after plasma treatment. Aquapel (Vulcavite) was introduced in the chip before usage to ensure hydrophobic channels walls. We compared various combinations of oils, surfactants, photoinitiators and UV dosages, as these are of paramount importance to obtain chondron-sized microgels. Chondron-sized droplets were produced by emulsifying a chondrocyte-laden hydrogel precursor solution of 10% (w/v) polyethylene glycol diacrylate (PEGDA, Laysan Bio, Inc.) and 0.1% (w/v) photoinitiator (Irgacure 2959, Ciba Specialty Chemicals) in hexadecane (Sigma) with 1% Span80 (Sigma). Subsequently, the microgels were formed in an on-chip delay channel by curing the emulsion with 365nm UV-light (Hamamatsu LC8).
**Results:** Chondrocytes were encapsulated in PEGDA microgels using droplet microfluidics at a rate of typically 20,000 cells per minute. The number of encapsulated cells followed a Poisson-distribution that was dependent on the cell concentration. We aimed to minimize the microgels' dimensions by comparing different combinations of oils, surfactants and UV dosages. Fluorinated oils in combination with PEGylated fluorosurfactants resulted in the most stable emulsions and thus allowed for the lowest photoinitiator concentration and UV dose for cell-laden microgel production. However, the use of fluorinated oils resulted in relatively large gels, which is a consequence of their low viscosity compared to the hydrogel precursor solution. In contrast, the use of a more viscous hydrocarbon oil with Span80 surfactant resulted in smaller, chondron-sized microgels. However, emulsions with this oil/surfactant combination were less stable off-chip. Solid on-chip cross-linking was accomplished by using an extended delay channel which enabled prolonged UV radiation without compromising the production rate. Finally, by varying the flow rates of the continuous and dispersed

phases, we were able to encapsulate single chondrocytes in stable monodisperse PEGDA microgels with a controlled diameter between 30 and 50 μm in a cell-friendly manner.
**Conclusions:** We have developed a microfluidic platform and optimized the production process for the encapsulation of single chondrocytes in chondron-sized microgels at a typical rate of 20,000 cells per minute. Future work will focus on the functionalization of the microenvironment within the biomaterial by means of incorporation of growth factors and extracellular matrix molecules to create biomimetic and chondrogenic artificial chondrons.

## 887
## OXYGEN IS A CRITICAL PARAMETER FOR CHONDROGENIC DIFFERENTIATION OF HUMAN UMBILICAL CORD BLOOD MESENCHYMAL STEM CELL IN 3D-CULTURES

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**Purpose:** The autologous chondrocyte implantation technique presents many limitations for cartilage disease treatment. The surgical process is traumatic and few chondrocytes are harvested. Mesenchymal stem cells (MSCs) represent a promising clinical tool for cartilage regeneration and osteoarthritis (OA) therapy. Although adult bone marrow has been up to now the main source of MSCs, their harvest is also a highly invasive procedure and their number, differentiation potential, and maximal life span decline with increasing age. The umbilical cord blood mesenchymal stem cells (UCB-MSCs) are a promising alternative source, due to abundant resources, painless and non-invasive collection process and are rapidly expandable in in vitro cultures. The aim of our study was to determine the chondrogenic differentiation potential of human UCB-MSCs for cartilage tissue engineering.
**Methods:** The UCB were collected after full term and normal delivery pregnancy. Cell amplification, karyotype, osteoblasts and adipocytes differentiation of UCB-MSCs were realized in normoxia. XTT cell assay was performed after 7 days under normoxic (21% O2) or hypoxic (< 5% O2) culture conditions. Cumulative population doublings and several surface markers of MSCs were analyzed up to the seventh passage. The chondrogenic differentiation was induced using an innovative approach combining 3D-culture in type I collagen sponges and chondrogenic factors (BMP-2 ± TGF-ß1) in normoxia or in hypoxia. Chondrogenic differentiation was then evaluated by real-time RT-PCR, Western-blotting and immunofluorescence microscopy after 7, 14 and 21 days of cell differentiation. The nature of the hyaline neo-tissue obtained after 14 days of in vitro differentiation was verified by subcutaneous implantation in the nude mice. The neo-tissue was collected after 35 days and histological examination was performed using hematoxylin-eosin-safran and alizarin red, as well as type I- and II-collagen immunohistochemistry analyses.
**Results:** 127 UCB samples were obtained with a mean volume of 41.15 ± 22.95 mL. Taking into account of only those with a minimal volume of 55 mL, the success efficiency was enhanced from 30% to 57%. Flow cytometry results showed that the isolated cells expressed CD29, CD44, CD73, CD90, CD105, CD166 and did not express CD14, CD34, CD45, CD64 and HLA-DR. There was no significant variation of those markers all along cell amplification. Otherwise, CD146 expression was shown to be donor- and passage-dependent. Interestingly, the growth of hypoxic UCB-MSC in monolayers induced the formation of cell spheres and a decrease in cell metabolism. Cells were shown to differentiate mainly in osteoblasts but not in adipocytes. The addition of BMP-2 and TGF-ß1 allowed to enhance chondrogenesis both in normoxia and hypoxia. Surprisingly, differentiation occurred earlier and at a higher level in normoxic compared to hypoxic conditions. These observations were confirmed by the analysis of 12 chondrogenic related genes and type II collagen protein. An increase in expression of specific cartilage markers (SOX9, COL2A1, COLIIB, ACAN et SNORC) and a slight MMP13 expression were observed during the culture. Otherwise, hypoxia presented beneficial effects by decreasing type X collagen. All the neo-tissue generated in vitro and injected in mice demonstrated that cells treated with BMP-2 and TGF-ß1 in normoxia showed a better phenotype maintenance with a more organized and abundant type II collagen matrix. Alizarin red staining was negative for all the donors.
**Conclusions:** Our experiments demonstrated that UCB-MSCs could be a reliable cell source for cartilage tissue engineering. In this sense, we suggest that UBC-MSCs cultured in in vitro 3D-culture can begin chondrogenesis in normoxic conditions to obtain a more efficient chondrogenic differentiation, before placing them in hypoxic conditions to stabilize the phenotype before in vivo implantation.

## 888
## BLOCK OF ANGIOGENESIS ENHANCES IN VIVO CHONDROGENESIS OF NASAL CHONDROCYTES-BASED CONSTRUCTS

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**Background/Aim:** Vascular Endothelial Growth Factor (VEGF) is involved in regulating cartilage growth and endochondral ossification during growth plate development, but also in degenerative processes e.g. in osteoarthritis or following mechanical overloading. On the other hand, VEGF expression is suppressed during hyaline cartilage formation and maintenance, and blocking of angiogenesis results in improved cartilage repair in murine models. We sought to investigate whether the sole blockade of the endogenous VEGF pathway could improve in vivo chondrogenesis in engineered tissues based on nasal chondrocytes (NC). Blockage of VEGF was achieved by transducing BMSC to express soluble VEGF receptor-2 (sFlk-1).
**Methods:** Human NCs were retrovirus-transduced to overexpress the soluble form of human VEGF receptor 2 (sFlk-1). Cell-based constructs were generated with either sFlk-1-expressing or control cells loaded on collagen-based scaffolds. To investigate their in vivo chondrogenic potential, the constructs were implanted in an ectopic mouse model. Vessel formation, VEGF activity, cartilage biochemistry and histology were performed to evaluate how the intrinsic chondrogenic capacity of NCs could benefit from blocking VEGF pathway.
**Results:** The released sFlk-1 blocked blood vessel ingrowth within the implant. Blood vessel ingrowth was significantly reduced in the engineered tissues developed by sFlk-1-expressing cells compared to control cells. sFlk-1 did not appear to regulate the in vitro chondrogenic differentiation of NCs. However, frank chondrogenesis in vivo could only be observed in the constructs generated by NCs expressing sFlk-1. Cartilaginous extracellular matrix was stable up to 8 weeks in vivo.
**Conclusion:** VEGF blockage was sufficient to induce in vivo chondrogenesis of NC in the absence of other exogenous morphogens. The long-term stability of the generated cartilage requires further investigations. The overexpression of sFlk-1 and the associated reduction in blood vessel ingrowth has profound effects on in vivo chondrogenesis by NCs, even in the absence of chondroinductive signals at the implant site. The absence of such effect in the in vitro model suggests a predominant paracrine mechanism, related to the control of angiogenesis by host cells.

## 889
## ARE UMBILICAL CORD MESENCHYMAL STEM CELLS ADVANTAGEOUS OVER BONE MARROW MESENCHYMAL STEM CELLS FOR CELL THERAPY IN ORTHOPAEDICS?

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**Purpose:** Mesenchymal Stem cells (MSC) from umbilical cords (UC) are of increasing interest for cell therapy in degenerative musculoskeletal disorders as they present little ethical consideration and are reported to contain immune privileged cells, which may be suitable for allogeneic based therapies. The use of MSCs as allogeneic cells *in vivo* would only be possible if they retained their immune privileged properties in an inflammatory environment. We have previously shown that cells obtained from UC are multipotent and have the recommended MSC cell surface marker profile according to the International Society for Cellular Therapy. The focus of this study was to characterise the immune properties of MSCs isolated from UC, particularly Wharton's jelly (WJ) and cells from whole cord (MC) as we have previously shown that cells from these cord regions have the best multipotency. MC and WJ were compared to Bone Marrow MSCs (BMSCs) in order to utilise the most promising population of stem cells and to analyse the immune properties of these cells before and after stimulation with the pro-inflammatory cytokine, interferon-γ (IFN-γ).