Conclusions: We have demonstrated clear differences in the regulation of chondrogenic potency and trophic properties of MSCs. The measurement of MMP13 gene and TIMP-1 protein markers will facilitate the design of future therapeutic strategies that exploit either of one these modes of action.

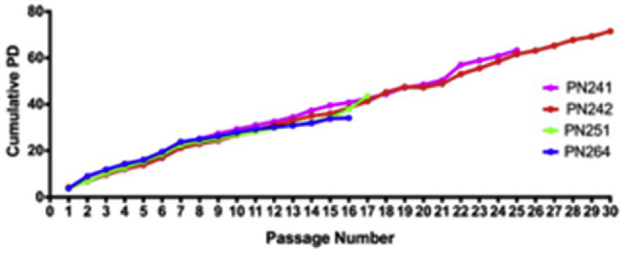

Figure 1. Population doublings at each passage for MSCs from four different patients

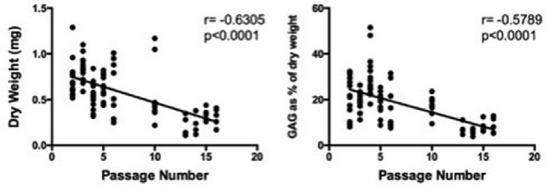

Figure 2. MSC Chondrogenesis measured as weight and GAG content of tissue engineered cartilage using cells from four different patients

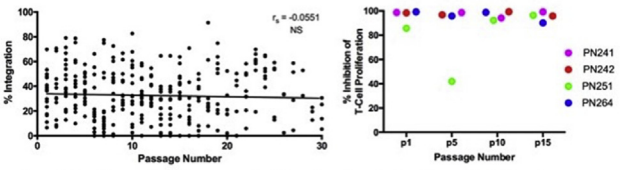

Figure 3. Trophic repair by MSCs measured as integration of meniscal cartilage and inhibition of T-Cell proliferation using cells from four different patients

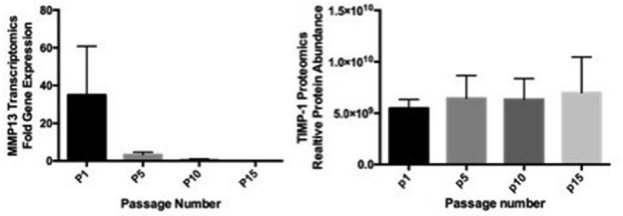

Figure 4. Changes in the MMP13 gene expression and TIMP-1 protein production with increasing passage using MSCs from four different patients

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AN *IN VITRO* STUDY TO INVESTIGATE THE EFFECTS OF STEM CELL THERAPY FOR TREATING OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a leading cause of chronic pain and disability, for which there is no cure. A range of non-operative treatments are used clinically, but only to manage symptoms until joint degeneration becomes so severe that an operation is performed to remove the diseased joint. Mesenchymal stem cells (MSCs) have recently brought new hope for treating OA due to their unique secretory functions, which send anti-inflammatory and trophic signals to the surrounding tissues. Interestingly, although the few available Phase I and II clinical trials utilising MSCs to treat knee OA have shown short-term effects in improving pain and joint function, they have not demonstrated sustained benefits. This may reflect a depletion of injected MSCs from the joint or a change/loss in their anti-inflammatory phenotype, and raises the question of whether repeated treatment by MSC injection will be beneficial.

Methods: We co-cultured bone marrow-derived human MSCs with human synovial fibroblasts (HSFs) isolated from osteoarthritic joint tissues. (1) To test whether osteoarthritic cells can modify the behaviour of MSCs, HSFs were co-cultured with human MSCs in growth, osteogenic and chondrogenic media, which simulated the types of conditions relevant for an osteoarthritic joint. (2) To test whether exposing osteoarthritic cells to MSCs can cause sustained changes in their behaviour and have positive effects on tissue repair, HSFs were either pre-conditioned by first co-culturing with MSCs for 3 days (+cHSF), or not pre-conditioned and simply cultured in growth medium (+HSF), and subsequently co-cultured with fresh MSCs in growth, osteogenic and chondrogenic media for 3 and 7 days. In both experiments, quantitative RT-PCR (n=4; mean ± SD) was used to evaluate inflammatory response in the MSCs and HSFs (IL-6, IL-8, MMP-2, MMP-13, ADAMTS4, ADAMTS5, CD44, TLR4, COX-2, CCL2), as well as differentiation in the MSCs (RUNX2, BSP, SPP1 for osteogenesis; SOX9, COL2A1, ACAN for chondrogenesis). In the first experiment, histology (n=2) was performed at 21 days to compare osteogenesis and chondrogenesis in MSCs between groups. Data was analysed using t-tests with $P<0.05$ considered statistically significant.

Results: MSCs co-cultured with osteoarthritic HSFs (Figure 1A) showed significant upregulation of several markers of inflammation, matrix degradation and tissue degeneration (e.g. MMP2, ADAMTS5, IL-8, CD44). They also showed significantly impaired ability to form new bone and cartilage, as evidenced by reduced expression of markers for osteogenesis (BSP, SPP1) and chondrogenesis (COL2A1, ACAN), and reduced histological features of differentiated bone (calcium) and cartilage (proteoglycan). These findings suggest that the osteoarthritic joint is a highly inhibitory environment that can increase inflammation in MSCs and significantly impair their regenerative ability. This explains clinical findings where MSCs did not have sustained therapeutic effects for knee OA.

Pre-conditioning the HSFs by exposing them to MSCs did not have any significant positive effects in modifying their behaviour (Figure 1B). HSFs, pre-conditioned or not, caused similar levels of inflammatory marker expression in MSCs in different media types and at different time points (e.g. ADAMTS4, IL-8, CD44). MSCs co-cultured with both pre-conditioned and non-pre-conditioned HSFs also showed impaired chondrogenesis to a similar extent (e.g. SOX9 expression, histology). Comparing the pre-conditioned and non-preconditioned HSFs, similar gene expression profiles were observed and pre-conditioning did not induce significant changes in the inflammatory phenotype of HSFs. These findings suggest that short-term exposure of osteoarthritic cells to MSCs is insufficient for sustained modifications to their diseased phenotype. Therefore, repeated MSC injections are unlikely to improve therapeutic efficacy in OA.

Conclusions: Although MSCs have anti-inflammatory and trophic functions, they could not provide long-term effects in correcting the osteoarthritic joint environment due to adopting the diseased phenotype of the surrounding tissues. Future regenerative therapies for OA that investigate the use of stem cells should shift focus away from testing different cell sources, injection concentrations and administration frequencies, and work on correcting the catabolic environment within the osteoarthritic joint, or utilise the biological derivatives of

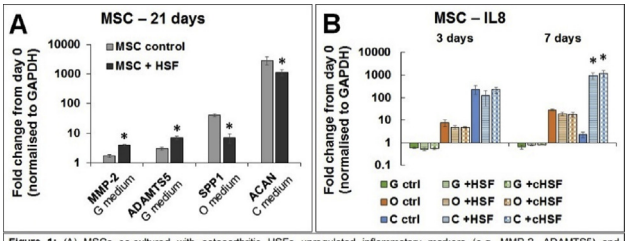

Figure 1: (A) MSCs co-cultured with osteoarthritic HSFs upregulated inflammatory markers (e.g. MMP-2, ADAMTS5) and downregulated osteogenic (e.g. SPP1) and chondrogenic (e.g. ACAN) markers (*$P < 0.01$). (B) HSFs, pre-conditioned or not, had similar effects on MSCs (e.g. IL-8; *$P < 0.001$). G=growth medium; O=osteogenic medium; C=chondrogenic medium.

stem cells which, unlike the living cell, will not respond in a negative way to the osteoarthritic environment.

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HYALURONIC ACID-BASED SHAPE MEMORY CRYOGEL SCAFFOLDS FOR FOCAL CARTILAGE DEFECT REPAIR

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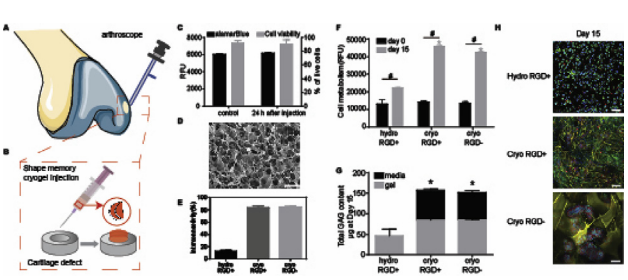
Purpose: Traumatic joint injuries can result in focal cartilage defects that regenerate poorly due to a lack of blood supply and low chondrocyte density. Cell-laden hydrogel scaffolds are under study but provide little flexibility in modulating their pore size, interconnectivity, swelling and stiffness, properties required for a conducive biomimetic environment. In this study, we engineered an injectable and biomimetic shape memory hyaluronic acid (HA)-based cryogel scaffolds with a macroporous and highly interconnected network in three-dimensions seeded with chondrocytes. Due to their unique ability to withstand up to 90% strains and rapidly recover their shape back, they can be arthroscopically delivered to fill up focal chondral defects for cartilage repair. Here we studied whether chondrocytes encapsulated within cryogels remain viable and metabolically active following injection and provide a superior micro-environment than conventional (nanoporous) hydrogels for cartilage regeneration. We also investigated how RGD-functionalized cryogel affects chondrocyte adhesion, migration and proliferation.
Methods: *Synthesis of HA-based Cryogels and Hydrogels:* HA cryogels were prepared using HA modified with glycidyl methacrylate (HAGM) and either Acryl-PEG-G4RGDS to synthesis gels functionalized with RGD (**Cryo RGD+**) or Acryl-PEG-methoxy to synthesize gels without RGD (**Cryo RGD-**) in deionized (DI) water. The mixture was cast in 6 mm diam x 1.5 mm cylindrical discs using APS/TEMED as our initiator system. The samples were frozen at -20°C and allowed to cryopolymerize overnight. Next, cryogels were brought to RT and washed/sanitized prior to cell seeding. HA hydrogels (**Hydro RGD+**) were fabricated via photopolymerization by mixing HAGM, RGD, and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photo-initiator in DI water. *Chondrocyte seeding and in-vitro culture:* Chondrocytes harvested from calf knee femoral condyle cartilage were seeded onto each scaffold and cultured for 15 days. *Injectability test:* Cell-laden cryogels were incubated overnight to enable complete cell adhesion and subsequently syringe injected through a 16-gauge needle before incubation for another 24h following which cell viability and metabolism were quantified. *Biochemical analysis:* At the end of 15-day culture, gel samples were digested to quantify the cell metabolism (Alamar Blue), DNA (PicoGreen) and GAG (DMMB) content. *Cell viability and cytoskeleton staining:* Gel samples were fixed and stained for nucleus, actin, dead cells and confocal imaged at 40x.
Results: Shape memory HA-based cryogels fully recovered their shape back following syringe injection through a 16-gauge needle without impacting cell metabolism or viability (Figs 1A-C). Scanning electron microscopy (SEM) images showed an average pore size of 49.2 ± 15 μm (Fig 1D) rendering a macroporous structure with high interconnectivity, which was 7x higher than hydrogels (Fig 1E). This unique macrostructural architecture enables efficient transport of nutrients and metabolites thereby offering a superior microenvironment for chondrocytes; both cell metabolism and GAG content was 2x higher in cryogels than hydrogels after 15-day culture (Figs 1F-G). Additionally, a significant amount of GAG (~70 μg) was released from both cryogels into media owing to their macroporous structure; no released GAG was measured in media from Hydro RGD+ (Fig 1G). The presence of RGD (cell-adhesion peptide) did not improve cell adhesion or biosynthesis rates. Chondrocytes formed larger organoid-like structures in Cryo RGD- while remaining homogenously dispersed in the presence of RGD in Cryo RGD+ (Fig 1H).
Conclusions: Syringe injectable HA-based shape memory cryogels provide a conducive microenvironment for chondrocyte adhesion, proliferation and matrix biosynthesis for use in repair of cartilage defects.



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MICRORNA PROFILE OF MESENCHYMAL STROMAL CELLS: A TOOL TO PREDICT THEIR THERAPEUTIC EFFICACY IN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a joint disease affecting > 5 million Canadians. Patients have limited palliative and joint-replacement surgical options, emphasizing the need for new curative therapies. Stromal cell therapy is emerging as a compelling treatment for OA. Our first-in-Canada Ph1/2 trial with bone marrow mesenchymal stromal cells (BM-MSCs) in OA patients showed significant improvements in patient outcomes. Pro-inflammatory monocytes/macrophages were reduced in the synovial fluid (SF), suggestive of a clinical MSC anti-inflammatory action. Although, MSCs showed beneficial effects in all the patients, we found variabilities in MSCs efficacy among participants. The goal of this study is to identify novel microRNAs (miRs) that correlate with therapeutic efficacy of MSCs in the context of OA.
Methods: We have identified a panel of predictive anti-inflammatory markers (i.e. IL10, HGF, IL6, TSG6, PGE2) on MSCs that is strongly indicative of clinical efficacy in OA. To expend this panel, we have conducted an unbiased miR sequencing on our clinical trial MSC samples exposed to pooled synovial fluid from eight OA patients in the same trial. We are correlating differential miR expression with patient outcomes.
Results: We have identified 25 miRs differentially expressed between MSCs from responder (5/ out of 5 KOOS sub-scale responses are clinically significant) and mild responder (2-3/5 KOOS sub-scale responses are clinically significant) participants. Amongst these, 12 miRs showed higher expression in MSCs from responder participants and 13 miRs appeared to be expressed at lower level when compared to those levels in MSCs from mild responder participants. Interestingly, we found that the identified miRs are associated with immune response, fibrosis, OA pathology and three linage differentiation of MSCs. Currently, we are verifying the miRs and their targets by qPCR to better understand and predict potent MSCs, and/or OA patients that are responders to MSC therapies.
Conclusions: microRNA profile of MSCs contributes to therapeutic efficacy of MSCs. Understanding therapeutically relevant mechanism of action of MSCs will help to develop enhanced MSCs; and define potency criterion for screening effective MSCs in OA patients. This in turn will enable a successful MSC pivotal clinical trial in OA.

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KRÜPPEL-LIKE FACTOR 10 IS A IMPORTANT MODULATORY FACTOR OF CHONDROCYTE HYPERTROPHY IN DEVELOPING SKELETON

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Purpose: To investigate the functional role of KLF10 as a modulator of chondrocyte hypertrophy in developmental skeleton. The developmental characteristics in the long bone of KLF10 knockout mice and characteristics of MSCs from KLF10 KO mice were investigated regarding chondrogenesis and osteogenesis. Delayed long bone growth and delayed formation of primary ossification center were observed in an early embryonic stage in KLF10 KO mouse along with very low Ihh expression in epiphyseal plate. While the chondrogenic potential of mMSCs appeared normal or slight decrerased in KLF10 KO mice, osteogenesis and hypertrophy were extensively suppressed. KLF10 was