expression levels of glycosaminoglycan (GAG) and target genes including wnt1, wnt3a, wnt5a, wnt10b, β-catenin, sox9, runx2, osteopontin, lipoprotein lipase, peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG), and collagen I, II, X mRNA on different concentrations of TGF-β1 were detect by ELISA assay and fluorescence quantitative (FQ)-PCR, respectively.
Results: After cultured in the TGF-β1 medium for 14 days, BMSCs differentiated into circular or polygonal cells. After 21 days, 95% of the cells were circular or polygonal cells with blue secretory granules within the cytoplasm by toluidine blue staining. Compared with the control group, the content of GAG increased significantly in the group of TGF-β1 medium, especially in 2ng/ml TGF-β1 group by ELISA method. FQ-PCR results indicated that mRNA expression level of wnt1, wnt3a, wnt5a, wnt10b and β-catenin after exposure to 2ng/ml TGF-β1 was obviously suppressed compared with control group, and mRNA expression level of sox9, collagen II was statistically enhanced, with an results of inhibiting expression of runx2, osteopontin, lipoprotein lipase, PPAR-γ, and collagen I, X. Moreover, we found that the expression of chondrogenic related genes was not upon a parallel line with the TGF-β1 concentration.
Conclusion: TGF-β1 can promote BMSCs differentiating into chondrocytes. During the differentiation progress, the expression of Wnt/β-catenin signaling pathway related genes was inhibited and chondrocyte specific genes of sox9, collagen II mRNA were increased. TGF-β may modulate chondrogenic differenciation though Wnt signaling pathway.

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CELLS ISOLATED FROM FAT PAD AND SYNOVIAL FLUID. ARE THEY SUITABLE FOR CARTILAGE REPAIR?

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Purpose: Autologous Chondrocyte Implantation (ACI) is used as a cellular therapy for treating defects in articular cartilage. Successful ACI depends on high cell number and quality of the cells which varies between patients. Alternative cell sources within the joint may provide a more preferable treatment for OA. The aim of this study is to evaluate the clinical suitability of infrapatellar fat pad (FP) and synovial fluid (SF) cells for cartilage repair by determining their MSC-like profile and response to an inflammatory stimulus in vitro.
Methods: Fat pad and synovial fluid were obtained with consent from the knees of patients undergoing ACI treatment. Cells were isolated from FP by enzymatic digestion with Collagenase I for 1 h at 37oC followed by centrifugation. Synovial fluid cells were obtained by centrifuging the synovial fluid. Resulting cell pellets were seeded onto tissue culture plastic in DMEM-F12, 10 % FCS and Penicillin/Streptomycin. Expression of cell surface markers was assessed using Flow cytometry (FACSCanto II). The multipotency of these cells was tested by culturing in monolayer in osteogenic and adipogenic media. Chondrogenesis was assessed in 3D pellet culture for 21 days. To evaluate the immunoresponsive nature of FP and SF cells, the expression of co-stimulatory markers CD40, CD80, CD86, and Major Histocompatibility complex II (HLA-DR) was tested before and after stimulation (48 h) with low (25ng/ml) and high (500ng/ml) concentrations of interferon-γ (IFN-γ). Results were compared to those obtained from bone marrow derived mesenchymal stem cells (BMSCs).
Results: SF and FP cells showed the ability to differentiate down osteogenic, adipogenic and chondrogenic lineages as shown by positive alkaline phosphatase (bone), Oil Red O (lipid) and Toluidine blue (glycosaminoglycan) staining.
Cells from both SF and FP were positive for the MSC markers CD73, CD90, CD105 and negative for HLA-DR. Following stimulation with IFN-γ, both SF and FP cells upregulated CD40 and HLA-DR. In comparison, BMSCs upregulated HLA-DR after IFN- γ stimulation but not the co-stimulatory marker CD40.
Conclusion: Cells isolated from FP and SF of osteoarthritic joints display immunogenic properties after stimulation with the pro-inflammatory cytokine IFN-γ which may make them unsuitable as alternative cell sources for ACI. Despite the production of co-stimulatory markers (CD40) and up regulation of HLA-DR as mentioned above, these cells do show multipotency via their ability to differentiate down osteogenic, adipogenic and chondrogenic lineages and they express the MSC markers CD73, 90 and 105. Their immunoresponsive nature needs to be studied further before these cells could be considered for routine applications for cartilage repair.

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DOES MACROPHAGE DEPLETION & CYTOKINE STIMULATION AFFECT THE PHENOTYPE OF SYNOVIAL MESENCHYMAL PROGENITOR CELLS IN VITRO?

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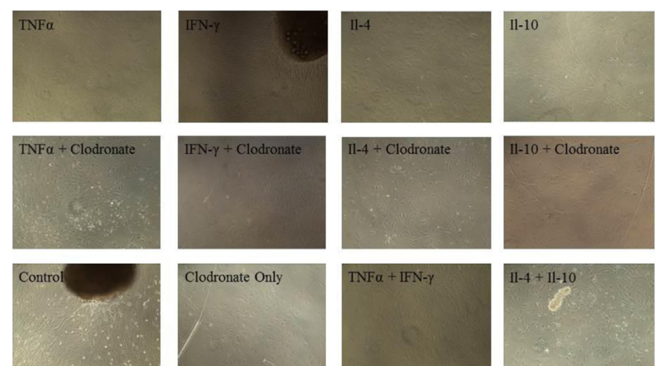
Purpose: The aim of the present study was to elucidate the relationship between synovial mesenchymal progenitor cells (sMPCs) and macrophages of the knee joint in OA pathogenesis. Specifically, this study sought to assess how components of the macrophage secretome (pro- and anti-inflammatory activation and polarization factors) affect the chondrogenic capacity of sMPCs in vitro. In addition to this, the effects of macrophage depletion from OA and normal biopsied human synovium were also assessed.
Methods: Patients with clinical and radiographic OA with no other co-morbidities consented and had synovial membrane biopsies obtained during knee arthroplasty or meniscal/ligamentous repair at the Peter Lougheed Centre, Canada. Synovial fluid and synovial membrane biopsies from macroscopically normal knees were obtained from cadavers less the 4 hrs after death. Tissue donors were received by the Southern Alberta Organ and Tissue Donation Program (SAOTDP). OA and normal biopsy samples were freshly plated and received 40ng/ml of IFN-γ, TNFα, Il-4, or IL-10, with or without Dichloromethylenediphosphonic acid disodium salt (Clodronate Disodium - Sigma) in solution every 4 days for 12 days post seeding (clodronate disodium is a first generation bisphosphonate utilized in research for the depletion of macrophages). Samples of the supernatant are also collected on days 4, 8, and 12 for proteomic assessment via Luminex. Following this treated 12 day outgrowth of sMPCs, cells are isolated, purified, expanded, and placed in 3 week chondrogenic differentiation (in pellet culture aggregates). qRT-PCR is conducted thereafter to assess gene expression levels of chondrogenic factors in addition to qualitative alcian blue staining.
Results: Our study revealed greater expression levels of Sox9, Col2a and Aggrecan on sMPCs which came from OA biopsy specimens that received the clodronate-only treatment in comparison to untreated biopsy specimens following chondrogenic differentiation. OA Biopsies which received anti-inflammatory cytokines (IL-10 & IL-4) during sMPC outgrowth also showed greater expression levels of Sox9, Col2a and Aggrecan following chondrogenic differentiation in comparison to those receiving pro-inflammatory cytokines (IFN-γ & TNFα). Additionally, OA biopsies receiving either pro-or anti-inflammatory cytokines alongside clodronate showed variable sMPC expression levels of chondrogenic factors following differentiation. The TNFα + Clodronate group showed the most reduced expression levels in comparison to sMPCs from untreated biopsies. Normal biopsies which underwent the same treatment regimens demonstrated similar results in the way of reduced chondrogenic gene expression following pro-inflammatory exposure, and increased expression following clodronate treatment.
Conclusions: Our findings shed light on macrophage effects on sMPC phenotype. Our results demonstrate that depletion of synovial macrophages increases the chondrogenic capacity of sMPCs and that pro-inflammatory cytokines which constitute an M1 macrophage secretome



reduce chondrogenic capacity of sMPCs. Taken together, these findings raise interesting insight into the inflammatory influences on OA pathogenesis and cartilage regeneration.

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MESENCHYMAL STEM CELLS IN SYNOVIAL FLUID INCREASE AFTER SYNOVIAL BIOPSY

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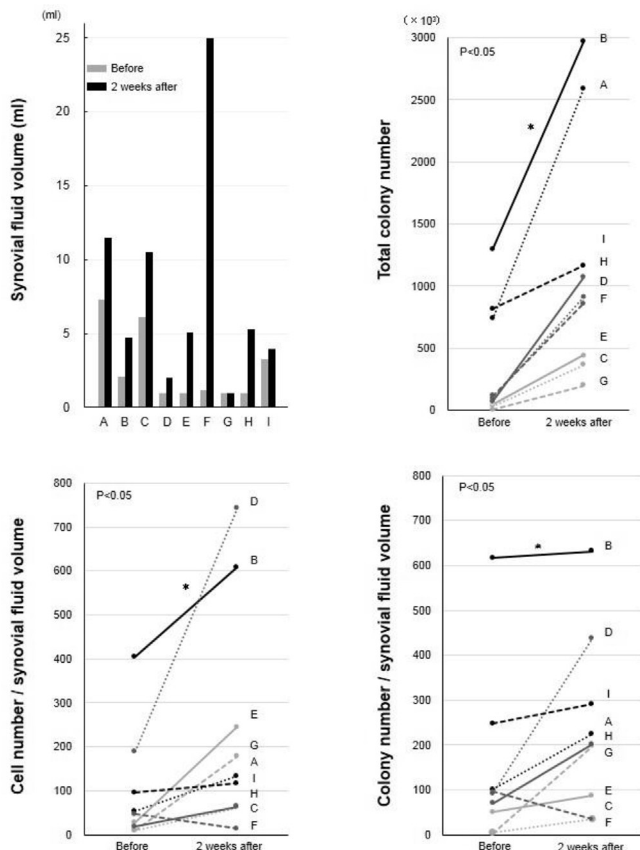
Purpose: We previously reported that the number of mesenchymal stem cells (MSCs) in synovial fluid was higher in knees with anterior cruciate ligament insufficiency and osteoarthritis than in normal knees. MSCs in synovial fluid were similar to synovium MSCs from the viewpoint of morphological and gene profiles. In this study, we investigated whether the number of MSCs in synovial fluid increased after synovial biopsy.
Methods: The study was approved by an institutional review board, and informed consents were obtained from all study subjects. We obtained synovial fluid from the knee joints of 9 patients, who underwent transplantation of synovial MSCs for cartilage injury, before and 2 weeks after harvest of 0.5g of synovium. We expanded the nucleated cells in synovial fluid for 2 weeks and counted the number of cell colonies stained with crystal violet. We also examined chondrogenic,adipogenic and osteogenic differentiation of these cells. For gene profile,28,000 genes were analyzed with microarray, and gene expressions were confirmed with quantitative real time-PCR.
Results: The total cell number obtained from 1ml of synovial fluid before synovial biopsy was 142 $\pm$ 192 and that 2 weeks after synovial biopsy was 238 $\pm$ 196. Total cell number obtained from 1ml of synovial fluid before synovial biopsy was $(95 \pm 129) \times 10^3$ and that 2 weeks after synovial biopsy was $(241 \pm 258) \times 10^3$.Both the number of cell colonies and the number of cells significantly increased after synovial biopsy ($p < 0.05$). The colony forming cells differentiated into chondrocytes, adipocytes, and osteoblasts. The expressions of subset of genes altered in MSCs derived from synovial fluid obtained before and after synovial biopsy.
Conclusion: MSCs in synovial fluid increased in the knee after synovial biopsy.
This was possibly due to inflammation and the repair process after synovial biopsy. It would be an interesting future topic to examine how many MSCs in synovial fluid contributed to the repair of the synovium.



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EXPRESSION ANALYSIS OF OSTEOARTHRITIS ASSOCIATED GENES IN MESENCHYMAL STEM CELLS DIFFERENTIATING INTO JOINT-ASSOCIATED CELLS

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Purpose: Historically recognised as a primary cartilage pathology, osteoarthritis (OA) is now understood to affect all joint-associated tissues. It has been hypothesised that gene dysregulation during joint formation may lead to the abnormal development and maintenance, or repair, of joint-associated tissues, thus conferring OA susceptibility. Genome wide association studies (GWAS) have identified multiple OA-associated genes, some of which have been shown to function during cell development and differentiation. However, many genes identified by such studies remain to be characterised. The first aim of this study was therefore to establish if a panel of OA-associated genes are expressed in mesenchymal stem cells (MSCs) that have undergone directed differentiation into joint-associated cells, and whether differential expression occurs in MSCs from OA patients. Additionally, this study sought to analyse the differentiation potential of OA patient-derived MSCs compared to MSCs from controls.
Methods: MSCs were extracted from the femoral head and from bone marrow aspirates of OA patients undergoing hip or knee arthroplasty respectively. Control MSCs were obtained from both neck of femur (NOF) fracture patients and young, healthy individuals. MSCs were subsequently differentiated using transwell culturing into osteoblasts, chondrocytes and adipocytes over a 14 day period, and RNA was extracted from cells harvested on days 0, 3 and 14 of differentiation. The differentiation potential of MSCs was analysed by histological staining and by quantitative PCR (qPCR) of lineage-specific genes. A selection of 13 genes within OA-susceptibility loci, as identified by GWAS, were studied during differentiation by qPCR.
Results: Osteogenic potential was preserved in OA patient-derived MSCs when compared to controls, as determined by Fast blue RR alkaline phosphatase staining and qPCR of osteogenic marker genes. Adipogenic differentiation, assessed by PPARγ induction, was found to be less efficient in OA patient MSCs, with decreased induction of PPARγ when compared to controls. qPCR of cDNA from chondrogenesis experiments revealed a large degree of inter-individual variability when analysing chondrogenic-specific genes, meaning it was not possible to deduce the chondrogenic efficiency in OA patient MSCs compared to controls. Of the genes contained within OA-associated regions, MCF2L was the only gene for which expression was not detected in any lineage during differentiation. The remaining 12 genes were expressed in a dynamic manner, with a large degree of inter-individual variability. Differential expression of individual genes was not apparent when comparing the OA and control groups.
Conclusions: Differentiation potential of OA-derived MSCs could be impaired during chondrogenesis and adipogenesis, while osteogenic differentiation appears to be preserved. To date, there are few published reports on the efficiency of OA-MSC chondrogenesis using transwell culture, which is believed to be a more faithful recapitulation of chondrogenesis when compared to other techniques. This may therefore have bearings on the success of autologous stem cell treatments for osteoarthritic patients, with further studies utilising more samples required to uphold this finding. MCF2L is not expressed in MSCs during in vitro differentiation into joint associated cells, which would suggest any potential effects of this gene on the OA joint is asserted later in development, or in the mature joint. All other genes within the OA-associated regions were expressed in a variety of patterns, indicating a possible function in joint development. However, no specific difference in expression was noted when comparing OA and control gene expression, meaning it was not possible to deduce specific genes of interest for future study. Despite this, the dynamic of expression of OA-associated genes in differentiating MSCs supports the possibility of a developmental aetiology of OA.