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 comorbidities 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