Determination of glycosaminoglycan (GAG) content: The synthesised GAG was determined by binding to DMB dye and the total amount of GAG was normalised to the amount of DNA by a PicoGreen dsDNA assay.
mRNA expression assay: Expressions of type I, II, X collagen, SOX-9, and aggrecan were analysed by real-time PCR. Expression of GAPDH was taken as an internal control.
Histologic and immunohistochemical examination: Pellets from each time point were stained with H&E and safranin-O for proteoglycan detection. The expression of type II collagen was detected by immunohistochemical (IHC) staining.
Results: Co-culture of human SDSCs and chondrocytes exhibited significantly higher GAG synthesis and type II collagen content. Furthermore, expression of type X collagen, a marker of MSC hypertrophy, was significantly lower in the co-culture pellets. The co-culture groups exhibited up-regulation of type II collagen and Sox-9 at seven days, and type I collagen, type X collagen, and aggrecan at 14 days. Safranin-O staining and IHC staining for type II collagen showed that chondrogenic differentiation of the co-culture group were higher than those of the chondrocytes or SDSCs single culture groups.
Discussion: Our results demonstrated that mixed co-culture of SDSCs and chondrocytes reduced cellular hypertrophy and enhance *in vitro* chondrogenesis when compared to the culture of chondrocytes or SDSCs alone.
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THE EFFECT OF LEUKOCYTE DEPLETION IN PRP ON THE PROLIFERATION AND CHONDROGENESIS OF SYNOVIUM-DERIVED MSCs AND EXPANDED CHONDROCYTES

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Introduction: Platelet-rich plasma (PRP) has been advocated as one treatment strategy for cartilage tissue regeneration. To date, several different platelet-rich formulations have been made available, but a deep knowledge of their composition and mechanism of action in a specific clinical use is needed. There are many variations in PRP formulations, with both platelet and leukocyte concentrations having been identified as major constituents affecting the inflammatory responses after PRP injection. The aim of this study was to investigate the effect of leukocyte depletion in PRP on the proliferation and chondrogenesis of synovium-derived MSCs (SD-MSCs) and chondrocytes.
Methods: Preparation of PRP formulations: From donated human fresh blood, we prepared three formulations of PRP: (1) platelet-poor plasma (PPP), (2) PRP with very few leukocytes (P-PRP), and (3) PRP with high concentrations of both platelets and leukocytes (L-PRP). PPP is the upper layer of plasma after centrifuging 10cc whole blood at 2400rpm for 10 minutes. After 2nd centrifuging at 3600rpm for 15 minutes, P-PRP is the lower layer of plasma without buffy coat. L-PRP is the lower layer of plasma with buffy coat.
Cell culture and *in-vitro* expansion: SD-MSCs and chondrocytes were obtained from patients undergoing total knee arthroplasty. The primary cells were expanded in DMEM with the three formulations. The cell proliferation was measured using the MTT assay.
Chondrogenesis: Chondrogenic differentiation of SD-MSCs and expanded chondrocytes were induced using a high-density pellet culture system. Aliquots of 5×10^5 cells were centrifuged at 500*g* for 5 minutes, which were subsequently cultured for three weeks. The pellets of SD-MSCs and chondrocytes were cultured in chemically defined chondrogenic medium with 10 ng/ml of TGF-β1 and/or 100 ng/ml of BMP-2.
Real-time PCR: Total RNA from SD-MSCs and chondrocytes were extracted by using the RNeasy mini kit (Qiagen). Expressions of type II collagen, type X collagen, Aggrecan, and Sox9 were analysed by RT-PCR. Expression of GAPDH was taken as the internal control.
Histological and immunohistochemical examination: Pellets were stained with Safranin-O for proteoglycan detection. The expression of type II collagen was detected by IHC staining and was observed under microscopy.
Results: Leukocyte depletion also lowered the count of platelets. L-PRP showed more proliferative effect on SD-MSCs and chondrocytes than P-PRP. RT-PCR revealed higher cartilage gene expression in TGF-β1 induced chondrogenesis of SD-MSCs and chondrocytes with P-PRP. However, there was a negative effect of both formulations in TGF-β1/BMP induced chondrogenesis. In TGF-β1 and/or BMP induced chondrogenesis, the SD-MSC pellet with P-PRP showed more proteoglycan production than those with L-PRP. Type II collagen synthesis in the chondrocyte pellets with P-PRP was greater than the pellets with L-PRP. However, type II collagen synthesis in SD-MSCs pellets was not detected.
Discussion: Although leukocyte depletion in PRP showed a negative effect on proliferation of SD-MSCs and chondrocytes and a positive effect on the mRNA expression of the chondrogenic differentiation marker genes, it could not change the negative effects of PRP on chondrogenic differentiation of SD-MSCs.
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DIFFERENCES IN MORPHOLOGY, PROLIFERATION, DIFFERENTIATION AND IMMUNE PROFILE AMONG SINGLE-CELL CLONED STEM CELLS FROM THE SAME MESENCHYMAL STEM CELL ORIGIN

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Introduction: Mesenchymal stem cells (MSCs) are believed to be immune-privileged due to lack of antigen-presenting-cell related markers; however, evidence suggests that MSCs are immunogenic and are attacked by the immune system. Our research investigates the hypothesis that there are differences between MSC clones from the same individual in terms of their morphology, proliferation, differentiation, and immune profile. Our goal is to discover immune-privileged stem cells, which can act as a universal allogenic mesenchymal stem cell donor to facilitate bone ingrowth for osteosarcoma patients' status post tumour excision and prosthesis implantation.
Subjects and Methods: Serial dilutions of bone-marrow derived (BMMSCs) and adipose derived mesenchymal stem cells (ADMSCs) from the same animal were carried out in order to isolate single-cell clones. From a single animal we obtained three clones from BMMSCs and three from ADMSCs. This procedure was repeated for another two animals. The proliferation rate and cell doubling time of each clonal culture was measured. The proliferation rate of mixed clonal cultures was also measured. The tri-differentiation potential of the clonal cultures was compared and a comparison was also made with the original isolates from bone marrow and fat. The immune-privileged properties were measured by flow cytometry and immuno-staining for the major histocompatibility complex (MHC) antigens. To measure the immune response a mixed leucocyte reaction was used but where leucocytes from a different individual were mixed with the clonal MSC cells.
Results: All isolates were able to differentiate into osteoblasts, chondrocytes, and adipocytes. All clonal cultures revealed significantly different proliferation rates and doubling times when compared with each other and with mixed cultures. All clonal cultures showed different surface marker presentations, which included differences in the expression of MHC antigens. One clone isolated from ADMSCs showed a lack of MHCI and MHCII. Our mixed leucocyte reaction and MHC staining showed a variety of immune-modulation and this was related to the expression of the MHC antigens.
Discussion and Conclusion: All clones tri-differentiated and therefore show a degree of 'stemness'. MSCs are generally believed not to express MHCII and to be immune-privileged. However, this study shows that the expression of these antigens in clones isolated from bone marrow and from fat is variable. A heterogeneous result indicates individual differences between MSCs, even from the same origin. The immune response elicited by MSCs is complicated. MSCs have been shown to release interleukin 10, which could inhibit the immune response but on the other hand interferon-gamma could enhance MHCII presentation in some MSCs. Our results confirmed our hypothesis because clonal cultures isolated from different sources of MSCs in the same animal show differences in proliferation rate, morphology, and surface marker presentation. Mesenchymal stem cells are not immunogenic or immune-privileged. Individual differences highlighted through single-cell clonal cultures may be the key to finding universal immune-privileged MSCs for allogeneic transplantation.
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IRON SUCROSE (VENOFER®) LABELLED ADIPOSE DERIVED STEM CELL BY USING 3T MAGNETIC RESONANCE IMAGING TRACKING: *IN VITRO* STUDY

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Background: Adipose derived stem cells (ASCs) based therapy is a promising strategy to promote in musculoskeletal repair. Cell differentiation ability, trophic factor and immunomodulation are considered as possible mechanisms to regenerate new tissue. However, the cell fate after implantation is required to understand treatment effects. In this study, we would like to investigate Iron sucrose labeling as the tool for MSC fate. Cell immunophenotypes, cell viability, cell differentiation ability after labelling are evaluated. Iron sucrose labelled MSCs were determined using 3 tesla (3T) MRI *in vitro*
Materials and methods: Adipose-derived mesenchymal stem cells (ASCs) were isolated from liposuction specimen from subcutaneous tissue. Passage 3rd ASCs were labelled with iron sucrose by adding in basal culture media (1 mg/ml) and