MSCs. proliferation of SD-MSCs and chondrocytes and a positive effect on the mRNA expression assay. 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 II collagen, a marker of MSC hypertrophy, was significantly lower in the co-culture pellets. The co-culture pellets exhibited up-regulation of type II collagen and 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. http://dx.doi.org/10.1016/j.jot.2016.06.120

414 THE EFFECT OF LEUKOCYTE DEPLETION IN PRP ON THE PROLIFERATION AND CHONDROGENESIS OF SYNOVIIUM-DERIVED MSCS AND EXPANDED CHONDRONCYES

Sahinghoon Lee, Hee Jung Park, Hyuk Soo Han, Bo Hyun Kim, Myung Chul Lee
Seoul National University College of Medicine, South Korea

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 10 minutes at 500 x g. PRP containing 108/mL of CB was used. Real-time PCR: Total RNA from SD-MSCs and chondrocytes were extracted by using the RNeasy mini kit (Qiagen). Expression of type II collagen, type X collagen, aggrecan, and Sox9 were analysed by RT-PCR. Expression of GAPDH was taken as an internal control. Results: Leukocyte depletion also lowered the count of platelets. L-PRP showed more proliferative effect on SD-MSCs and chondrocytes than PPP. RT-PCR revealed higher cartilage gene expression in TGF-β1 induced chondrogenesis of SD-MSCs and chondrocytes with L-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 L-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-MSC 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.

http://dx.doi.org/10.1016/j.jot.2016.06.122

473 IRON SUCROSE (VENOFER®) LABELLED ADIPOSE DERIVED STEM CELL BY USING 3T MAGNETIC RESONANCE IMAGING TRACKING: IN VITRO STUDY

Paweens Tangchitphisut*, Sith Phongkitkarun b, Naronrit Sirkaew c, Tulyapruek Tawonsawatrak x
xDepartment of Orthopaedics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand
bDepartment of Radiology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand
cOffice of Research and Innovation, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand

Background: Adipose derived stem cells (ASCs) based therapy is a promising strategy to promote in musculoskeletal repair. Cell differentiation ability, trophic factors 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 labeling 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 using 3 tesla (3T) MRI in vitro.

http://dx.doi.org/10.1016/j.jot.2016.06.121

465 DIFFERENCES IN MORPHOLOGY, PROLIFERATION, DIFFERENTIATION AND IMMUNE PROFILE AMONG SINGLE-CELL CLONED STEM CELLS FROM THE SAME MESENCHYMAL STEM CELL ORIGIN

Yenwen Cheng, Sorousheh Samizadeh, Melanie Coathup, Gordon Blunn
University College London, United Kingdom

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 (BM-MSCs) and adipose derived mesenchymal stem cells (AD-MSCs) from the same animal were carried out in order to isolate single-cell clones. From a single animal we obtained three clones from BM-MSCs and three from AD-MSCs. 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 MSCs.

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 AD-MSCs showed a lack of MHC1 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.

http://dx.doi.org/10.1016/j.jot.2016.06.122

Session: Regenerative Medicine

108