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MENISCUS REGENERATION BY SYNGENEIC, MINOR MISMATCHED, AND MAJOR MISMATCHED TRANSPLANTATION OF SYNOVIAL MESENCHYMAL STEM CELLS IN A RAT MODEL

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Mesenchymal stem cells (MSCs), especially those derived from synovium, are an attractive cell source for meniscus regeneration, because synovial MSCs have remarkable proliferation and chondrogenic potential. We previously reported that intraarticular injection of synovial MSCs promoted meniscal regeneration in a rat model. It was a syngeneic transplantation model; therefore, possible immune reactions seem to be negligible. We compared the effect of syngeneic and allogeneic transplantation of synovial mesenchymal stem cells (MSCs) for meniscus regeneration in a rat model.

Synovium was harvested from the knee joints of 3 strains of rats, F344, Lewis, and ACI. The anterior half of the medial meniscus in both knees of F344 rats was removed and 5 million synovial MSCs of 3 strains were injected into the right knee of F344 rats. F344 MSC transplantation is regarded as a syngeneic model, Lewis MSC transplantation as a minor mismatched model, and ACI MSC transplantation as a major mismatched model.

At 4 weeks, the area of the regenerated meniscus in the F344 and Lewis groups was significantly larger than that in the ACI group ($n = 5$; $p < 0.05$). Histological score was significantly better in the F344 group than in the ACI group at 4 weeks ($n = 5$; $p < 0.05$), and significantly better in the F344 and Lewis groups than in the ACI group at 8 weeks ($n = 4$; $p < 0.05$). One week after DiI labeled MSCs were transplanted, DiI positive area in the syngeneic group was larger than in the ACI group. The number of ED1 positive macrophage in the synovium significantly increased in the ACI group than in the F344 group ($n = 4$; $p < 0.05$). The number of CD8 positive T cells in the synovium also increased in the ACI group than in the F344 group ($n = 4$; $p < 0.05$).

Syngeneic and minor mismatched transplantation of synovial MSCs promoted meniscus regeneration better than major mismatched transplantation in a rat meniscectmized model. Synovial MSCs were rejected by immune reaction in a major mismatched transplantation. If allogeneic synovial MSCs are used for meniscal regeneration in a clinical situation, their histocompatibility antigens should be closer to those of the recipient.

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PELVIS IS SUPERIOR TO FEMUR AND TIBIA AS A SOURCE FOR MINIMALLY MANIPULATED MESENCHYMAL STEM CELLS

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Purpose: Since their description in 1999 mesenchymal stem cells (MSCs) has been intensively investigated for the treatment of a variety of musculoskeletal conditions. Whilst a large amount of pre-clinical work has been undertaken this has yet to be translated into a widely used or accepted clinical treatment for use in orthopedic conditions. The evolving nature of our understanding of MSCs and their actions means that there is also a lack of information as to the mechanism of action of some of the effects of MSCs that have been demonstrated. The high cost of laboratory based techniques and developing knowledge about the paracrine actions of MSCs has led to increased interest in the use of so-called single sitting orthopedic procedures where MSCs are harvested from an individual and re-implanted at the site of injury in one sitting.

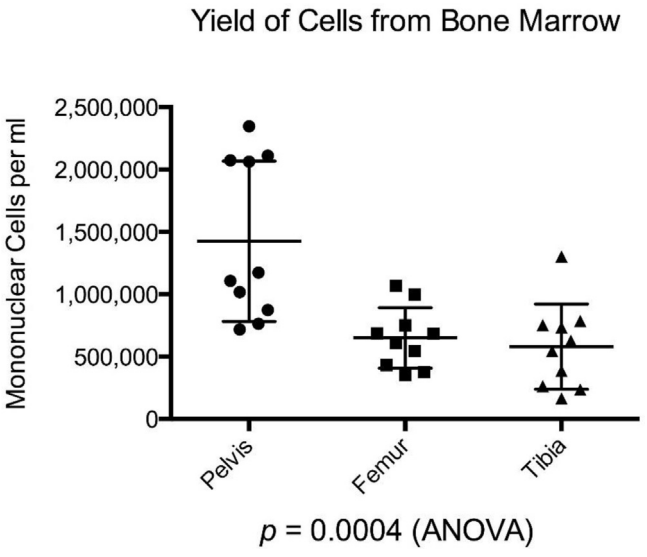
In orthopedic surgery the obvious site to harvest MSCs from is bone marrow given the ease of access for orthopedic surgeons. However, the relatively low number of MSCs found in bone marrow means that yields are low. Previous work has demonstrated that MSCs can be harvested from a variety of different bones including the pelvis, femur, humerus, tibia, and calcaneus. The pelvis is currently the gold standard site for harvesting bone marrow for use in procedures such as cartilage repair of the knee. However, harvesting from the pelvis results in a second operative site and thus increased morbidity. We have therefore sought to determine if bone marrow harvested from either the tibia or the femur can provide MSCs in comparable amounts to that obtained from the pelvis.

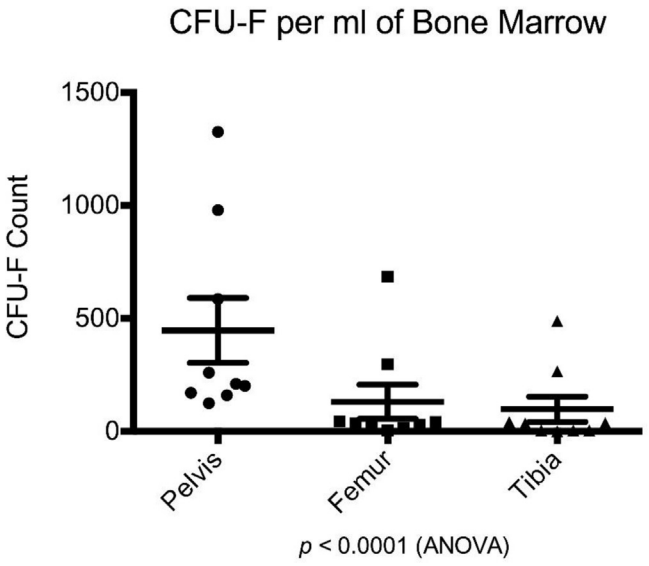
Methods: Samples were obtained from patients undergoing knee arthroplasty. Participants provided informed consent and approval was obtained from the local Research Ethics Committee. An 8G Jamshidi® biopsy needle and syringe were used to obtain bone marrow samples from the pelvis, femur, and tibia of each individual. Samples were taken before any surgical procedure was carried out on the long bones. A maximum of 10 mls of bone marrow was sampled to avoid dilution of the bone marrow with peripheral blood. Samples were placed in EDTA coated tubes and transported to the laboratory immediately.

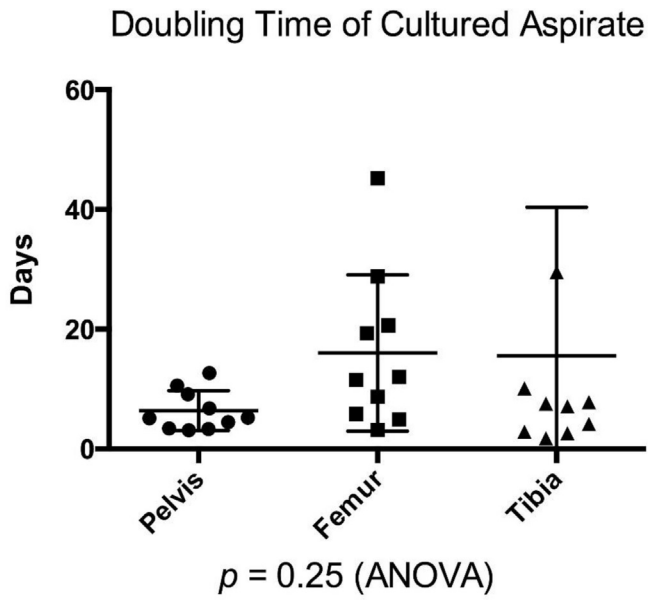
The mononuclear cellular layer was extracted using a density centrifugation technique. A total cell count was then performed using a Millipore Scepter^{TM}. 2 x 10^6 cells were removed from the sample to undertake colony-forming unit- fibroblast (CFU-F) count assessments in duplicate and the remaining cells seeded into a tissue culture flask. All cells were fed with Invitrogen MesenPro RS^{TM} media every 2-3 days.

CFU-F cells were fixed at day 14 and stained with Giemsa stain to allow colony counting. Colonies were counted if they were >1mm in diameter. Cells were passaged when they reached 70-80% confluence and population-doubling information was recorded at each passage up to the end of passage three.

Results: The graphs below show the initial cell yield, CFU-F count, and sample doubling time for the first 10 individuals (Male to female ratio 6:4, mean age = 64.2 yrs, range = 52 - 77 yrs). N is less than 10 in some groups due to failure of cells to expand in culture. Missing data for 4 individuals for doubling times (where one site failed to grow in culture) was imputed using the multiple imputation by chained equations method in Stata 12. All other statistical analyses was carried out using GraphPad Prism 6. ANOVA tests showed a significant difference ($p < 0.05$) in initial cell yield and CFU-F count between the different sites sampled but no significant difference in doubling times. These results show that despite the presence of MSCs in all bone marrow the samples obtained from the pelvis were superior in both number of cells obtained and CFU-F count.







Conclusions: The pelvis would appear to remain the optimum site for harvesting of bone marrow for use in single sitting procedures despite the increased morbidity from harvesting at this site. Further work is now being undertaken to establish the properties of MSCs from these different sites in terms of their ability to differentiate into desired tissues such as cartilage and bone.

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MESENCHYMAL STROMAL CELLS ENCAPSULATION IN INNOVATIVE BIOMATERIALS: APPLICATION TO OSTEOARTHRITIS TREATMENT

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Osteoarthritis (OA) is a very common disease affecting a growing part of aging population. It is a degenerative joint disease accompanied by degradation of the articular cartilage and variable degrees of synovium inflammation. Mesenchymal Stromal Cells (MSC) have generated significant medical consideration since they secrete immuno-modulatory and anti-inflammatory factors. Unfortunately, the intra-articular injection of MSC suffers some major limitations including: (i) a massive cell death upon injection in the articular space making difficult to detect the injected cells for a sustained time; (ii) a risk of cell leak outside the articular space due to the propensity of MSC to migrate.

Purpose: The present project is devoted to the development of a therapeutic strategy for OA, based on the exploitation of the immunomodulatory properties of MSC. To overcome the limitations of the crude intraarticular injections (cell death and leakage of MSC), we propose to entrap bioactive MSC prior to their injection within cytoprotective and permeable microcapsules made of innovative biomaterials.

Materials: Alginate and silylated hydroxypropyl methylcellulose (Si-HPMC) were chosen as biocompatible biomaterials able to support the viability and bioactivity of encapsulated MSC. MSC were isolated from human adipose tissue (hADSC: human adipose tissue stromal cells).

We selected a dropwise method in CaCl2 solution to obtain alginate capsules. To produce SI-HPMC microcapsules, we developed water in oil (w/o) emulsion protocol with or without surfactant. To assess pore size of microcapsules, the diffusion of FITC-dextran molecules (sizes ranging from 20 to 2000 KDa) through alginate and Si-HPMC microcapsules were followed by confocal microscopy. For MSC encapsulation, a hADSC suspension of 2.106 cells/ml was added either to the alginate solution or to the Si-HPMC solution (without surfactant). The microbeads were then collected by filtration, washed with HEPES buffer and seeded in culture medium. Culture medium was changed every 2 days after cell encapsulation. hADSC viability after encapsulation in alginate and Si-HPMC was followed for a period of 24 h to 2 months using a Live/Dead Viability/Cytotoxicity kit.

Results: By dropwise method, we obtained alginate capsules with an average size of 1,1 ±0,2 mm. Preliminary results have shown that their pore size was between 10 nm and 21 nm.

With Si-HPMC, we managed to develop a suitable microencapsulation method by varying operating parameters such as surfactant, temperature or rotating speed. In optimal conditions, we obtained Si-HPMC microbeads with a size of 50,3 ±4,9 μm (when a surfactant was used) and 1 ±0,9 mm (without surfactant). Their pore size was ranging from 10 nm to 21 nm.

Then we encapsulated hADSC in both polymers. We detected a high rate of viable encapsulated cells in 1 mm alginate capsules (about 93% of viability at 2 months post-encapsulation). In addition, we observed a lower rate of viable encapsulated hADSC in 1 mm Si-HPMC capsules (less than 50% of viability at 48 h post-encapsulation).

Conclusions: Alginate and Si-HPMC appear as suitable biomaterials for producing permeable microcapsules. Dropwise and emulsion methods allow us to obtain capsules with different sizes. Our data strongly suggest that capsule porosity is appropriate to maintain cell viability (diffusion of nutrients and oxygen) and biological functions. Further experiments are now under investigation to determine whether hADSC encapsulated in permeable biomaterials may be a relevant strategy to prevent cartilage degradation and inflammation in OA.

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EFFECTS OF TRANSFORMING GROWTH FACTOR β1 ON THE EXPRESSION OF WNT SIGNALING PATHWAY RELATED GENES IN THE CHONDROGENIC DIFFERENTIATION OF BONE MARROW STROMAL STEM CELLS

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Objective: Osteochondral defects is a very common and chanllenging problem of orthopaedic surgery. The mature chondrocyte embedded in the avascular (no blood vessels) and aneural (no neurons and nerves) articular cartilage lacking of usual self-regeneration (the process of repair by formation of the same type of tissue) after injury or disease. Stromal stem cells (MSCs) are multipotent cells and able to differenciate into chondrocytes under certain culture medium (such as TGF-β, BMPs, IGFs) and easily proliferate while maintaining their undifferentiated state. Previous researches have proved that Wnt/β-catenin signaling pathway is essential for stem cells self-renewal and chondrogenic differenciation. In this study, we intend to investigate the transforming growth factor-β1 (TGF-β1) on expression of Wnt signaling pathway related genes during the chondrogenesis of bone marrow stem cells (BMSCs) in vitro.

Methods: The BMSCs of immature SPF SD rat, isolated and cultured with or without TGF-β1 (2ng/ml TGF-β1 medium containing 10% fetal bovine serum) in vitro. Cultured cells were observed by inverted phase contrast microscope and identified by toluidine blue staining. The