**Monomeric, porous type II collagen scaffolds promote chondrogenic differentiation of human bone marrow mesenchymal stem cells in vitro.**

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**Abstract**

Osteoarthritis (OA) is a common cause of pain and disability and is often associated with the degeneration of articular cartilage. Lesions to the articular surface, which are thought to progress to OA, have the potential to be repaired using tissue engineering strategies; however, it remains challenging to instruct cell differentiation within a scaffold to produce tissue with appropriate structural, chemical and mechanical properties. We aimed to address this by driving progenitor cells to adopt a chondrogenic phenotype through the tailoring of scaffold composition and physical properties. Monomeric type-I and type-II collagen scaffolds, which avoid potential immunogenicity associated with fibrillar collagens, were fabricated with and without chondroitin sulfate (CS) and their ability to stimulate the chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells was assessed. Immunohistochemical analyses showed that cells produced abundant collagen type-II on type-II scaffolds and collagen type-I on type-I scaffolds. Gene expression analyses indicated that the addition of CS - which was released from scaffolds quickly - significantly upregulated expression of type II collagen, compared to type-I and pure type-II scaffolds. We conclude that collagen type-II and CS can be used to promote a more chondrogenic phenotype in the absence of growth factors, potentially providing an eventual therapy to prevent OA.

**Oscillatory fluid flow induces the osteogenic lineage commitment of mesenchymal stem cells: The effect of shear stress magnitude, frequency, and duration.**

Stavenschi E\(^1\), Labour MN\(^1\), Hoey DA\(^2\).

**Abstract**

A potent regulator of bone anabolism is physical loading. However, it is currently unclear whether physical stimuli such as fluid shear within the marrow cavity is sufficient to directly drive the osteogenic lineage commitment of resident mesenchymal stem cells (MSC). Therefore, the objective of the study is to employ a systematic analysis of oscillatory fluid flow (OFF) parameters predicted to occur in vivo on early MSC osteogenic responses and late stage lineage commitment. MSCs were exposed to OFF of 1Pa, 2Pa and 5Pa magnitudes at frequencies of 0.5Hz, 1Hz and 2Hz for 1h, 2h and 4h of stimulation. Our findings demonstrate that OFF elicits a positive osteogenic response in MSCs in a shear stress
magnitude, frequency, and duration dependent manner that is gene specific. Based on the mRNA expression of osteogenic markers Cox2, Runx2 and Opn after short-term fluid flow stimulation, we identified that a regime of 2Pa shear magnitude and 2Hz frequency induces the most robust and reliable upregulation in osteogenic gene expression. Furthermore, long-term mechanical stimulation utilising this regime, elicits a significant increase in collagen and mineral deposition when compared to static control demonstrating that mechanical stimuli predicted within the marrow is sufficient to directly drive osteogenesis.


**Visualization of Mesenchymal Stromal Cells in 2D and 3D Cultures by Scanning Electron Microscopy with Lanthanide Contrasting.**

Novikov IA, Vakhrushev IV, Antono EN, Yarygin KN, Subbot AM.

**Author information**

**Abstract**

Mesenchymal stromal cells from deciduous teeth in 2D- and 3D-cultures on culture plastic, silicate glass, porous polystyrene, and experimental polylactoglycolide matrices were visualized by scanning electron microscopy with lanthanide contrasting. Supravit staining of cell cultures with a lanthanide-based dye (neodymium chloride) preserved normal cell morphology and allowed assessment of the matrix properties of the carriers. The developed approach can be used for the development of biomaterials for tissue engineering.


**Exosomes Secreted from Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Prevent Osteonecrosis of the Femoral Head by Promoting Angiogenesis.**


**Author information**

**Abstract**

**Background:** Local ischemia is the main pathological performance in osteonecrosis of the femoral head (ONFH). There is currently no effective therapy to promote angiogenesis in the femoral head. Recent studies revealed that exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells (iPS-MSC-Exos) have great therapeutic potential in ischemic tissues, but whether they could promote angiogenesis in ONFH has not been reported, and little is known regarding the underlying mechanism. **Methods:** iPS-MSC-Exos were intravenously injected to a steroid-induced rat osteonecrosis model. Samples of the femoral head were obtained 3 weeks after all the injections. The effects were assessed by measuring local angiogenesis and bone loss through histological and immunohistochemical (IHC) staining, micro-CT and three-dimensional microangiography. The effects of exosomes on endothelial cells were studied through evaluations of proliferation, migration and tube-
forming analyses. The expression levels of angiogenic related PI3K/Akt signaling pathway of endothelial cells were evaluated following stimulation of iPS-MSC-Exos. The promoting effects of exosomes were re-evaluated following blockade of PI3K/Akt. Results: The in vivo study revealed that administration of iPS-MSC-Exos significantly prevented bone loss, and increased microvessel density in the femoral head compared with control group. We found that iPS-MSC-Exos significantly enhanced the proliferation, migration and tube-forming capacities of endothelial cells in vitro. iPS-MSC-Exos could activate PI3K/Akt signaling pathway in endothelial cells. Moreover, the promoting effects of iPS-MSC-Exos were abolished after blockade of PI3K/Akt on endothelial cells. Conclusions: Our findings suggest that transplantation of iPS-MSC-Exos exerts a preventative effect on ONFH by promoting local angiogenesis and preventing bone loss. The promoting effect might be attributed to activation of the PI3K/Akt signaling pathway on endothelial cells. The data provide the first evidence for the potential of iPS-MSC-Exos in treating ONFH.


Osteo Growth Induction titanium surface treatment reduces ROS production of mesenchymal stem cells increasing their osteogenic commitment.

Ghensi P1, Bressan E2, Gardin C3, Ferroni L3, Ruffato L4, Caberlotto M5, Soldini C6, Zavan B6.

Author information

Abstract

Surface characteristics play a special role for the biological performance of implants and several strategies are available to this end. The OGI (Osteo Growth Induction) titanium surface is a surface, obtained by applying a strong acid onto the blasted surface. The aim of this in-vitro study is to evaluate in vitro the osteoproperties of OGI surfaces on Mesenchymal Stem cells derived from dental pulp. Our results confirm that this treatment exert a positive effect on mitochondrial homeostasis, as shown by a decrease in ROS production related to environmental stress on the mitochondria. Morphological and molecular biology analyses confirmed more over that the DPSC cultured on the OGI surfaces appeared more spread in comparison to those grown on control titanium surface and real time PCR and biochemical data clearly demonstrated the increase of osteoconductive properties of the OGI treatment. In conclusion, our results suggest that mesenchymal stem cells sensitively respond to surface properties related to OGI treatment enhancing their osteogenic activities.


Irradiation of Mesenchymal Stromal Cells with Low and High Doses of Alpha Particles Induces Senescence and/or Apoptosis.

Alessio N1, Esposito G2,3, Galano G4, De Rosa R5, Anello P2, Peluso G6, Tabocchini MA2,3, Galderisi U7,1,6.

Author information

Abstract
The use of high-linear energy transfer charged particles is gaining attention as a medical tool because of the emission of radiations with an efficient cell-killing ability. Considerable interest has developed in the use of targeted alpha-particle therapy for the treatment of micrometastases. Moreover, the use of helium beams is gaining momentum, especially for treating pediatric tumors. We analyzed the effects of alpha particles on bone marrow mesenchymal stromal cells (MSCs), which have a subpopulation of stem cells capable of generating adipocytes, chondrocytes, and osteocytes. Further, these cells contribute toward maintenance of homeostasis in the body. MSCs were irradiated with low and high doses of alpha particles or X-rays and a comparative biological analysis was performed. At a low dose (40 mGy), alpha particles exhibited a limited negative effect on the biology of MSCs compared with X-rays. No significant perturbation of cell cycle was observed, and a minimal increase in apoptosis or senescence was detected. Self-renewal was preserved as revealed by the CFU assay. On the contrary, with 2000 mGy alpha particles we observed adverse effects on the vitality, functionality, and stemness of MSCs. These results are the consequence of different proportion of cells targeted by alpha particles or X-rays and the quality of induced DNA damage. The present study suggests that radiotherapy with alpha particles may spare healthy stem cells more efficaciously than X-ray treatments, an observation that should be taken into consideration by physicians while planning irradiation of tumor areas close to stem cell niches, such as bone marrow. This article is protected by copyright. All rights reserved.


Intratendinous adipose-derived stromal vascular fraction (SVF) injection provides a safe, efficacious treatment for Achilles tendinopathy: results of a randomized controlled clinical trial at a 6-month follow-up.


Author information
Abstract

PURPOSE:
Although platelet-rich plasma (PRP) injection has shown controversial results for the treatment of Achilles tendinopathy, it remains the most used biological treatment. Recent findings seem to demonstrate that the stromal vascular fraction (SVF) within adipose tissue may counteract the impaired tendon homeostasis. The aim of this study was to prospectively compare the efficacy of PRP and SVF injection for the treatment of non-insertional Achilles tendinopathy.

METHODS:
Fourty-four patients were recruited in the study; 23 of them were assigned to the PRP group whereas 21 to the SVF group, treated unilaterally or bilaterally for a total of 28 tendons per group. All patients (age 18-55 years) were clinically assessed pre-operatively and at 15, 30, 60, 120 and 180 days from treatment, using the VAS pain scale, the VISA-A, the AOFAS Ankle-Hindfoot Score and the SF-36
form. The patients were also evaluated by ultrasound and magnetic resonance before treatment and after 4 (US only) and 6 months.

RESULTS:
Both treatments allowed for a significant improvement with respect to baseline. Comparing the two groups, VAS, AOFAS and VISA-A scored significantly better at 15 and 30 days in the SVF in comparison to PRP group (p < 0.05). At the following time points the scores were not significantly different between the two groups. No correlation has been found between clinical and radiological findings.

CONCLUSIONS:
Both PRP and SVF were safe, effective treatments for recalcitrant Achilles tendinopathy. The patients treated with SVF obtained faster results, thus suggesting that such a treatment should be taken into consideration for those patients who require an earlier return to daily activities or sport.


Challenges of bone tissue engineering in orthopaedic patients.
Guerado E¹, Caso E¹.
Author information
Abstract
Bone defects may impede normal biomechanics and the structural stability of bone as an organ. In many cases, the correction of bone defects requires extensive surgical intervention involving the use of bone-grafting techniques and other procedures in which healing is slow, there is a high risk of infection and considerable pain is provoked - with no guarantee of complete correction of the defect. Therefore, the search for surgical alternatives continues to present a major challenge in orthopaedic traumatology. The reamer-irrigator-aspirator (RIA) system, which was devised to avoid the problems that can arise with autograft harvesting from the iliac crest, consists of collecting the product of the femoral canal after reaming. The RIA technique improves osteogenic differentiation of mesenchymal stem cells, compared to bone marrow aspiration or cancellous bone harvesting from the iliac crest using a spoon. Another approach, the Masquelet technique, consists of reconstructing a long bone defect by means of an induced membrane grown onto an acrylic cement rod inserted to fill the defect; in a second surgical step, once the membrane is constituted, the cement rod is removed and cancellous autograft is used to fill the defect. Both in RIA and in the Masquelet technique, osteosynthesis is usually needed. Bone transportation by compression-distraction lengthening principles is commonly implemented for the treatment of large bone loss. However, complications are frequently encountered with these techniques. Among new techniques that have been proposed to address the problem of large bone loss, the application of stem cells in conjunction with tissue engineering techniques is very promising, as is the creation of personalised medicine (or precision medicine), in which molecular profiling technologies are used to tailor the therapeutic strategy, to ensure the right method is applied for the right person at the right time, after determining the predisposition to disease among the general population. All of the above techniques for addressing bone defects are discussed in this paper.
Osteogenesis and Mineralization of Mesenchymal Stem Cells in Collagen Type I Based Recombinant Peptide Scaffolds.

Pawelec KM, Confalonieri D, Ehlicke F, van Boxtel HA, Walles H, Kluijtmans SG.

Abstract
Recombinant peptides have the power to harness the inherent biocompatibility of natural macro-molecules, while maintaining a defined chemistry for use in tissue engineering. Creating scaffolds from peptides requires stabilization via cross-linking, a process known to alter both mechanics and density of adhesion ligands. The chemistry and mechanics of linear scaffolds from a recombinant peptide based on human collagen type I (RCP) was investigated after cross-linking. Three treatments were compared: dehydrothermal treatment (DHT), hexamethylene diisocyanate (HMDIC), and genipin. With cross-linking, mechanical properties were not significantly altered, ranging from 1.9 - 2.7 kPa. However, the chemistry of the scaffolds was changed, affecting properties such as water uptake, and initial adhesion of human mesenchymal stem cells (hMSCs). Genipin cross-linking supported the lowest adhesion, especially during osteoblastic differentiation. While significantly altered, RCP scaffold chemistry did not affect osteoblastic differentiation of hMSCs. After four weeks in vitro, all scaffolds showed excellent cellular infiltration, with up-regulated osteogenic markers (RUNX2, Osteocalcin, Collagen type I) and mineralization, regardless of the cross-linker. Thus, it appears that, without significant changes to mechanical properties, cross-linking chemistry did not regulate hMSC differentiation on scaffolds from recombinant peptides, a growing class of materials with the ability to expand the horizons of regenerative medicine.

Osteogenic Lineage Commitment of Adipose-Derived Stem Cells is Predetermined by Three-Dimensional Cell Accumulation on Micropatterned Surface.

Furuhata Y, Yoshimoto K, Yoshitomi T, Kikuchi Y, Sakao M.

Abstract
Lineage commitment of stem cells is mainly regulated by their microenvironments, which comprise soluble growth factors, extracellular matrix, mechanical forces, and cell density. Although numerous studies have investigated stem cell response to these factors in two-dimensional (2D) culture, little is known about that in 3D culture. Here, we studied effects of 3D cell accumulation levels on the differentiation behavior of mesenchymal stem cells (MSCs) by using a micropatterned surface. After induction of 3D-cultured MSCs on the surface, their osteogenic differentiation was significantly promoted, while adipogenic differentiation was not. This differentiation behavior of densely packed MSCs in 3D culture is unlike that in 2D culture. Moreover, to determine the contributing factor of this commitment, the relationship between 3D cell accumulation levels and their differentiation potential was studied before differentiation induction. A series of MSCs with varied 3D accumulation levels was constructed on the micropatterned surface, where the accumulated MSCs were not in hypoxic
environment. Interestingly, with increasing 3D accumulation levels, MSCs enhanced their osteogenic potential but repressed adipogenic potential in gene expression level. These results suggest that preconditioned 3D microenvironments with high cell accumulation levels promote osteogenic differentiation of MSCs and their accumulation levels help in regulating MSC differentiation.


**Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model.**

**Joswig AJ¹, Mitchell A¹, Cummings KJ², Levine GJ³, Gregory CA⁴, Smith R 3rd³, Watts AE⁵.**

**Author information**

**Abstract**

**BACKGROUND:**
Intra-articular injection of mesenchymal stem cells (MSCs) is efficacious in osteoarthritis therapy. A direct comparison of the response of the synovial joint to intra-articular injection of autologous versus allogeneic MSCs has not been performed. The objective of this study was to assess the clinical response to repeated intra-articular injection of allogeneic versus autologous MSCs prepared in a way to minimize xeno-contaminants in a large animal model.

**METHODS:**
Intra-articular injections of bone marrow-derived, culture-expanded MSCs to a forelimb metacarpophalangeal joint were performed at week 0 and week 4 (six autologous; six autologous with xeno-contamination; six allogeneic). In the week following each injection, clinical and synovial cytology evaluations were performed.

**RESULTS:**
Following the first intra-articular injection, there were no differences in clinical parameters over time. Following the second intra-articular injection, there was a significant adverse response of the joint to allogeneic MSCs and autologous MSCs with xeno-contamination with elevated synovial total nucleated cell counts. There was also significantly increased pain from joints injected with autologous MSCs with xeno-contamination.

**CONCLUSIONS:**
Repeated intra-articular injection of allogeneic MSCs results in an adverse clinical response, suggesting there is immune recognition of allogeneic MSCs upon a second exposure.

**KEYWORDS:**
Bone marrow; FBS; Fetal bovine serum; Fetal calf serum; Flare; Ho

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**Effects of Hypoxia in Long Term In Vitro Expansion of Human Bone Marrow Derived Mesenchymal Stem Cells.**
INTRODUCTION:
Mesenchymal stem cells (MSC) are considered multipotent stromal, non-hematopoietic cells with properties of self-renovation and differentiation. Optimal conditions for culture of MSC have been under investigation. The oxygen tension used for cultivation has been studied and appears to play an important role in biological behavior of mesenchymal cells. The aim is characterize MSC in hypoxia and normoxia conditions comparing their morphological and functional characteristics.

METHODS:
Bone marrow-derived mesenchymal stem cells obtained from 15 healthy donors and cultured. MSC obtained from each donor were separated into two cultivation conditions normoxia (21% O₂) and hypoxia (three donors at 1%, three donors at 2%, five donors at 3%, and four donors at 4% O₂) up to second passage. MSC were evaluated for proliferation, differentiation, immunophenotyping, size and cell complexity, oxidative stress, mitochondrial activity, and autophagy.

RESULTS:
Culture conditions applied didn't seem to affect immunophenotypic features and cellular plasticity. However, cells subjected to hypoxia showed smaller size and greater cellular complexity, besides lower proliferation (p < 0.002). Furthermore, cells cultured in low O₂ tension had lower mitochondrial activity (p < 0.03) and a reduced tendency to autophagy, although oxidative stress didn't vary among groups (p < 0.39).

CONCLUSION:
Oxygen tension seems to be a key regulator of cellular adaptation in vitro, and metabolic effects underlying this variable remain undescribed. Heterogeneity or even lack of results on the impact of oxygen concentration used for expanding MSC highlights the need for further research, in order to optimize conditions of cultivation and expansion and achieve greater safety and therapeutic efficacy.

Engineering a humanized bone organ model in mice to study bone metastases.

Current in vivo models for investigating human primary bone tumors and cancer metastasis to the bone rely on the injection of human cancer cells into the mouse skeleton. This approach does not mimic species-specific mechanisms occurring in human diseases and may preclude successful clinical
translation. We have developed a protocol to engineer humanized bone within immunodeficient hosts, which can be adapted to study the interactions between human cancer cells and a humanized bone microenvironment in vivo. A researcher trained in the principles of tissue engineering will be able to execute the protocol and yield study results within 4-6 months. Additive biomanufactured scaffolds seeded and cultured with human bone-forming cells are implanted ectopically in combination with osteogenic factors into mice to generate a physiological bone 'organ', which is partially humanized. The model comprises human bone cells and secreted extracellular matrix (ECM); however, other components of the engineered tissue, such as the vasculature, are of murine origin. The model can be further humanized through the engraftment of human hematopoietic stem cells (HSCs) that can lead to human hematopoiesis within the murine host. The humanized organ bone model has been well characterized and validated and allows dissection of some of the mechanisms of the bone metastatic processes in prostate and breast cancer.