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Cartilage and bone tissue engineering using adipose stromal/stem cells spheroids as building blocks.

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Author information

Abstract

Scaffold-free techniques in the developmental tissue engineering area are designed to mimic *in vivo* embryonic processes with the aim of biofabricating, *in vitro*, tissues with more authentic properties. Cell clusters called spheroids are the basis for scaffold-free tissue engineering. In this review, we explore the use of spheroids from adult mesenchymal stem/stromal cells as a model in the developmental engineering area in order to mimic the developmental stages of cartilage and bone tissues. Spheroids from adult mesenchymal stromal/stem cells lineages recapitulate crucial events in bone and cartilage formation during embryogenesis, and are capable of spontaneously fusing to other spheroids, making them ideal building blocks for bone and cartilage tissue engineering. Here, we discuss data from ours and other labs on the use of adipose stromal/stem cell spheroids in chondrogenesis and osteogenesis *in vitro*. Overall, recent studies support the notion that spheroids are ideal "building blocks" for tissue engineering by "bottom-up" approaches, which are based on tissue assembly by advanced techniques such as three-dimensional bioprinting. Further studies on the cellular and molecular mechanisms that orchestrate spheroid fusion are now crucial to support continued development of bottom-up tissue engineering approaches such as three-dimensional bioprinting.

PLoS One. 2020 Mar 12;15(3):e0229914. doi: 10.1371/journal.pone.0229914. eCollection 2020.

Extracellular vesicles from rat-bone-marrow mesenchymal stromal/stem cells improve tendon repair in rat Achilles tendon injury model in dose-dependent manner: A pilot study.

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Abstract

Mesenchymal stromal/stem cells (MSCs) are increasingly employed for tissue regeneration, largely mediated through paracrine actions. Currently, extracellular vesicles (EVs) released by MSCs are major mediators of these paracrine effects. We evaluated whether rat-bone-marrow-MSC-derived EVs (rBMSCs-EVs) can ameliorate tendon injury in an in vivo rat model. Pro-collagen1A2 and MMP14 protein are expressed in rBMSC-EVs, and are important factors for extracellular-matrix tendon-

remodeling. In addition, we found pro-collagen1A2 in rBMSC-EV surface-membranes by dot blot. In vitro on cells isolated from Achilles tendons, utilized as rBMSC -EVs recipient cells, EVs at both low and high doses induce migration of tenocytes; at higher concentration, they induce proliferation and increase expression of Collagen type I in tenocytes. Pretreatment with trypsin abrogate the effect of EVs on cell proliferation and migration, and the expression of collagen I. When either low- or high-dose rBMSCs-EVs were injected into a rat-Achilles tendon injury-model (immediately after damage), at 30 days, rBMSC-EVs were found to have accelerated the remodeling stage of tendon repair in a dose-dependent manner. At histology and histomorphology evaluation, high doses of rBMSCs-EVs produced better restoration of tendon architecture, with optimal tendon-fiber alignment and lower vascularity. Higher EV-concentrations demonstrated greater expression of collagen type I and lower expression of collagen type III. BMSC-EVs hold promise as a novel cell-free modality for the management of tendon injuries.

Stem Cells Int. 2020 Feb 15;2020:5045124. doi: 10.1155/2020/5045124. eCollection 2020.

Modulation of Adipose-Derived Mesenchymal Stem/Stromal Cell Transcriptome by G-CSF Stimulation.

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Abstract

Mesenchymal stem/stromal cells (MSCs) exhibit multidifferentiation potential, paralleled with immunomodulatory and trophic properties that make them viable alternative tools for the treatment of degenerative disorders, allograft rejection, autoimmune diseases, and tissue regeneration. MSC functional attributes can be modulated by exposing them to inflammatory-stimulating microenvironments (*i.e.*, priming) before their therapeutic use. Granulocyte-colony stimulating factor (G-CSF) is a cytokine that plays key roles in immune response and hematopoiesis modulation through direct effects on hematopoietic progenitors' proliferation, survival, and mobilization. Despite the established roles of MSCs supporting hematopoiesis, the effects of G-CSF on MSCs biology have not been thoroughly explored. This study reveals that G-CSF has also direct effects on adipose-derived MSCs (ADSCs), modulating their functions. Herein, microarray-based transcriptomic analysis shows that G-CSF stimulation *in vitro* results in modulation of various signaling pathways including ones related with the metabolism of hyaluronan (HA), conferring a profile of cell mobilization to ADSCs, mediated in a cell-intrinsic fashion in part by reducing CD44 expression and HA synthesis-related genes. Collectively, these data suggest a direct modulatory effect of G-CSF on ADSC function, potentially altering their therapeutic capacity and thus the design of future clinical protocols.

Stem Cell Res Ther. 2020 Mar 20;11(1):129. doi: 10.1186/s13287-020-01635-5.

Administration of allogeneic mesenchymal stem cells in lengthening phase accelerates early bone consolidation in rat distraction osteogenesis model.

Yang Y[1,2], Pan Q[2,3], Zou K[1,2], Wang H[2,3], Zhang X[2,3], Yang Z[2,3], Lee WYW[2,3], Wei B[4], Gu W[1], Yang YP[5,6,7], Lin S[8,9,10,11], Li G[12,13,14,15].

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Abstract

BACKGROUND:

Distraction osteogenesis (DO) is a surgical technique to promote bone regeneration which may require long duration for bone consolidation. Bone marrow-derived mesenchymal stem cells (MSCs) have been applied to accelerate bone formation in DO. However, the optimal time point for cell therapy in DO remains unknown. This study sought to determine the optimal time point of cell administration to achieve early bone consolidation in DO. We hypothesized that the ratio of circulating MSCs to peripheral mononuclear cells and the level of cytokines in serum might be indicators for cell administration in DO.

METHODS:

Unilateral tibial osteotomy with an external fixator was performed in adult Sprague Dawley rats. Three days after osteotomy, the tibia was lengthened at 0.5 mm/12 h for 5 days. At first, 5 rats were used to analyze the blood components at 6 different time points (3 days before lengthening, on the day lengthening began, or 3, 6, 10, or 14 days after lengthening began) by sorting circulating MSCs and measuring serum levels of stromal cell-derived factor 1 (SDF-1) and interleukin 1β. Then, 40 rats were used for cell therapy study. A single dose of 5×10^5 allogeneic MSCs was locally injected at the lengthening site on day 3, 6, or 10 after lengthening began, or 3 doses of MSCs were injected at the three time points. Sequential X-ray radiographs were taken weekly. Endpoint examinations included micro-computed tomography analysis, mechanical testing, histomorphometry, and histology.

RESULTS:

The number of circulating MSCs and serum level of SDF-1 were significantly increased during lengthening, and then decreased afterwards. Single injection of MSCs during lengthening phase (on day 3, but not day 6 or 10) significantly increased bone volume fraction, mechanical maximum loading, and bone mineralization of the regenerate. Triple injections of MSCs at three time points also significantly increased bone volume and maximum loading of the regenerates.

CONCLUSION:

This study demonstrated that bone consolidation could be accelerated by a single injection of MSCs during lengthening when the ratio of peripheral MSCs to mononuclear cells and the serum SDF-1 presented at peak levels concurrently, suggesting that day 3 after lengthening began may be the optimal time point for cell therapy to promote early bone consolidation.

Stem Cell Res Ther. 2020 Mar 19;11(1):125. doi: 10.1186/s13287-020-01641-7.

Chemical-defined medium supporting the expansion of human mesenchymal stem cells.

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Abstract

OBJECTIVES:

Mesenchymal stem cells (MSCs) have been intensively investigated as to their therapeutic potentials. However, the full chemical-defined medium supporting the isolation and expansion of human MSCs has not been developed yet.

MATERIALS AND METHODS:

Here, we developed the full chemical-defined medium, NBVbe medium, via RNA sequencing, bioinformatic analysis, and growth factor screening.

RESULTS:

The NBVbe medium contains N2B27 medium with the BSA (bovine serum albumin) replaced by the recombinant human albumin, bFGF (basic fibroblast growth factor), vitamin C, and EGF (epidermal growth factor). The NBVbe medium could support the isolation and expansion of human MSCs from the umbilical cords.

CONCLUSIONS:

The full chemical-defined medium supporting the isolation and expansion of human MSCs has been developed. This would be helpful for further optimization of the MSC medium, their clinical applications, and molecular characterization.

Respir Res. 2020 Mar 20;21(1):71. doi: 10.1186/s12931-020-1331-4.

Mesenchymal stromal cell-derived exosomes improve pulmonary hypertension through inhibition of pulmonary vascular remodeling.

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Abstract

BACKGROUND:

Pulmonary hypertension (PH) is a life-threatening disease characterized by pulmonary vascular remodeling, right ventricular hypertrophy and failure. So far no effective treatment exists for this disease; hence, novel approaches are urgently needed. The aim of the present research was to observe the treatment effect of mesenchymal stromal cell derived exosomes and reveal the mechanism.

METHODS:

Monocrotaline (MCT)-induced PH in rats and hypoxia-induced cell damage model were established, respectively. Exosomes derived from the supernatant of human umbilical cord mesenchymal stem cells (MSC-exo) were injected into MCT-PH model rat or added into the cells cultured medium.

Immunohistochemistry, quantitative real-time polymerase chain reaction (qRT-PCR) and western blot methods were used in vivo and vitro.

RESULTS:

The results showed that MSC-exo could significantly attenuate right ventricular (RV) hypertrophy and pulmonary vascular remodelling in MCT-PH rats. In the cell culture experiments, we found that MSC-exo could significantly inhibit hypoxia-induced pulmonary arterial endothelial cell (PAEC) apoptosis and pulmonary arterial smooth muscle cells (PASMC) proliferation. Furthermore, the pulmonary arterioles endothelial-to-mesenchymal transition (EndMT) was obviously suppressed. Moreover, the present study suggest that MSC-exo can significantly upregulate the expression of Wnt5a in MCT-PH rats and hypoxic pulmonary vascular cells. Furthermore, with Wnt5a gene silencing, the therapeutic effect of MSC-exo against hypoxia injury was restrained.

CONCLUSIONS:

Synthetically, our data provide a strong evidence for the therapeutic of MSC-exo on PH, more importantly, we confirmed that the mechanism was associated with up-regulation of the expression of Wnt5a. These results offer a theoretical basis for clinical prevention and treatment of PH.

Biomed Res Int. 2020 Mar 3;2020:6094562. doi: 10.1155/2020/6094562. eCollection 2020.

Proteomic Analysis of Exosomes from Adipose-Derived Mesenchymal Stem Cells: A Novel Therapeutic Strategy for Tissue Injury.

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Abstract

Exosomes are extracellular membranous nanovesicles that mediate local and systemic cell-to-cell communication by transporting functional molecules, such as proteins, into target cells, thereby affecting the behavior of receptor cells. Exosomes originating from adipose-derived mesenchymal stem cells (ADSCs) are considered a multipotent and abundant therapeutic tool for tissue injury. To investigate ADSC-secreted exosomes and their potential function in tissue repair, we isolated exosomes from the supernatants of ADSCs via ultracentrifugation, characterized them via transmission electron microscopy, nanoparticle tracking analysis, and Western blot analysis. Then, we determined their protein profile via proteomic analysis. Results showed that extracellular vesicles, which have an average diameter of 116 nm, exhibit a cup-shaped morphology and express exosomal markers. A total of 1,185 protein groups were identified in the exosomes. Gene Ontology analysis indicated that exosomal proteins are mostly derived from cells mainly involved in protein binding. Protein annotation via the Cluster of Orthologous Groups system indicated that most proteins were involved in general function prediction, posttranslational modification, protein turnover, and chaperoning. Further, pathway analysis revealed that most of the proteins obtained participated in metabolic pathways, focal adhesion, regulation of the actin cytoskeleton, and microbial metabolism. Some tissue repair-related signaling

pathways were also discovered. The identified molecules might serve as potential therapeutic targets for future studies.

 Front Bioeng Biotechnol. 2020 Feb 28;8:148. doi: 10.3389/fbioe.2020.00148. eCollection 2020.

Five Decades Later, Are Mesenchymal Stem Cells Still Relevant?

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Abstract

Mesenchymal stem cells are culture-derived mesodermal progenitors isolatable from all vascularized tissues. In spite of multiple fundamental, pre-clinical and clinical studies, the native identity and role in tissue repair of MSCs have long remained elusive, with MSC selection *in vitro* from total cell suspensions essentially unchanged as a mere primary culture for half a century. Recent investigations have helped understand the tissue origin of these progenitor cells, and uncover alternative effects of MSCs on tissue healing *via* growth factor secretion and interaction with the immune system. In this review, we describe current trends in MSC biology and discuss how these may improve the use of these therapeutic cells in tissue engineering and regenerative medicine.

J Tissue Eng Regen Med. 2020 Mar 15. doi: 10.1002/term.3032. [Epub ahead of print]

Platelet-Rich Plasma counteracts detrimental effect of high glucose concentrations on Mesenchymal Stem Cells from Bichat fat pad.

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Abstract

Diabetic patients display increased risk of periodontitis and failure in bone augmentation procedures. Mesenchymal stem cells (MSCs) and platelet rich plasma (PRP) represent a relevant advantage in tissue repair process and regenerative medicine. We isolated MSCs from Bichat's buccal fat pad (BFP) and measured the effects of glucose and PRP on cell number and osteogenic differentiation potential. Cells were cultured in presence of 5.5 mM glucose (Low glucose; LG) or 25 mM glucose (High glucose; HG). BFP-MSC number was significantly lower when cells were cultured in HG compared with those in LG. Following osteogenic differentiation procedures, calcium accumulation, alkaline phosphatase activity and expression of osteogenic markers were significantly lower in HG compared with LG. Exposure of BFP-MSC to PRP significantly increased cell number and osteogenic differentiation potential, reaching comparable levels in LG and in HG. Thus, high glucose concentrations impair BFP-

MSC growth and osteogenic differentiation. However, these detrimental effects are largely counteracted by PRP.

Ann Vasc Surg. 2020 Mar 12. pii: S0890-5096(20)30194-1. doi: 10.1016/j.avsg.2020.03.001. [Epub ahead of print]

Different drugs effect on mesenchimal stem cells isolated from abdominal aortic aneurysm.

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Abstract

INTRODUCTION:

Abdominal aortic aneurysm (AAA) is a progressive dilation of the aortic wall, determined by the unbalanced activity of matrix metalloproteinase (MMPs). In vitro and in vivo studies support the pivotal role of MMP-9 to AAA pathogenesis. In our experience, we elucidated the expression of MMP-9 in an ex-vivo model of human Mesenchymal Stem Cells isolated from AAA specimen (AAA-MSCs). Thus, MMP-9 inhibition could be an attractive therapeutic strategy for inhibiting AAA degeneration and rupture. Our study was aimed at testing the effect of three different drugs (pioglitazone, doxycycline, simvastatin) on MMP-9 and PPAR-γ expression in AAA-MSCs.

METHODS:

Aneurysmal aortic wall segments were taken from AAA patients after the open surgical treatment. MSCs were isolated from AAA (n=20) tissues through enzymatic digestion. AAA-MSCs were exposed to different doses of pioglitazone (5-10-25 μM), doxycycline (10-25 μM) and simvastatin (10 μM) for 24 h. The effect of each drug was evaluated in terms of cell survival, by crystal violet stain. MMP-9 and PPAR- γ mRNA were analysed by Real Time PCR.

RESULTS:

AAA-MSCs were not affected by the exposure to the selected drugs, as shown by the analysis of cell viability. Interestingly, MMP-9 mRNA resulted significantly decreased after each treatment, recording a down-regulation of 50% in presence of pioglitazone, 90% with doxycycline and 40% with exposed to simvastatin, in comparison to untreated cells. We further analysed the expression of PPAR-γ, target of pioglitazone, observing an up-regulation in exposed AAA-MSCs to controls.

CONCLUSIONS:

Our data support the potential therapeutic effect of pioglitazone, doxycycline and simvastatin on AAA by reducing the MMP-9 expression in a patient-specific model (AAA-MSCs). In addition, pioglitazone drives the increase of PPAR-G, another promising target for AAA therapy. Further studies are necessary to elucidate the mechanism driving this inhibitory pathway, which can reduces the mortality risk associated with AAA rupture.

Curr Osteoporos Rep. 2020 Mar 14. doi: 10.1007/s11914-020-00572-9. [Epub ahead of print]

Skeletal Stem Cells for Bone Development and Repair: Diversity Matters.

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Abstract

PURPOSE OF REVIEW:

Skeletal stem cells (SSCs) are considered to play important roles in bone development and repair. These cells have been historically defined by their in vitro potential for self-renewal and differentiation into "trilineage" cells; however, little is known about their in vivo identity. Here, we discuss recent progress on SSCs and how they potentially contribute to bone development and repair.

RECENT FINDINGS:

Bone is composed of diverse tissues, which include cartilage and its perichondrium, cortical bone and its periosteum, and bone marrow and its trabecular bone and stromal compartment. We are now at the initial stage of understanding the precise identity of SSCs in each bone tissue. The emerging concept is that functionally dedicated SSCs are encased by their own unique cellular and extracellular matrix microenvironment, and locally support its own compartment. Diverse groups of SSCs are likely to work in concert to achieve development and repair of the highly functional skeletal organ.

Mol Med Rep. 2020 Mar 12. doi: 10.3892/mmr.2020.11025. [Epub ahead of print]

Mouse bone marrow mesenchymal stem cells with distinct p53 statuses display differential characteristics.

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Author information

Abstract

Mesenchymal stem cells (MSCs) affect diverse aspects of tumor progression, such as angiogenesis, tumor growth and metastasis. Bone marrow MSCs (BM-MSCs) are fibroblast-like cells with multipotent differentiation ability, that localize to areas of tissue damage, including wounds and solid tumors. The tumor suppressor gene, p53, is functionally involved in cell cycle control, apoptosis and genomic stability, and is mutated and inactivated in most human cancers. The present study aimed to investigate the role of p53 in the biology of BM-MSCs. In the present study, p53 wild-type (p53+/+), knockdown (p53+/-) and knockout (p53-/-) mouse BM-MSCs (mBM-MSCs) were observed to be similar in appearance and in the expression of cell surface biomarkers, but expressed differential p53 protein levels. The p53+/- and p53-/- mBM-MSCs demonstrated an increased proliferation rate compared with mBM-MSCs derived from p53+/+ mice. mBM-MSCs from all three groups, representing distinct p53 statuses, were unable to form tumors over a 3-month period in vivo. The adipogenic and osteogenic differentiation of mBM-MSCs was increased in the absence of p53. The colony formation and migratory abilities of p53+/- and p53-/- mBM-MSCs were markedly enhanced, and the expression levels of stem cell-associated proteins were significantly increased compared with p53+/+. The expression levels of

microRNA (miR)-3152 and miR-337 were significantly increased in p53+/- and p53-/- mBM-MSCs, whereas the expression levels of miR-221, miR-155, miR-1288 and miR-4669 were significantly decreased. The expression levels of tumor necrosis factor-α and interferon-γ-inducible protein-10 were significantly upregulated in the supernatant of p53+/- and p53-/- mBM-MSCs. Ubiquitin protein ligase E3 component n-recognin 2, RING-finger protein 31 and matrix metalloproteinase 19 were highly expressed in p53+/- and p53-/- mBM-MSCs. The results of the present study indicated that p53 may serve an important role in the biology of mBM-MSCs, and may provide novel insights into the role of cells with different p53 statuses in cancer progression.