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Colloids Surf B Biointerfaces. 2019 Jan 24;177:68-76. doi: 10.1016/j.colsurfb.2019.01.050. [Epub ahead of print]

Biomimetic microspheres for 3D mesenchymal stem cell culture and characterization.

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Author information

Abstract

Stem cells reside in niches, specialized microenvironments that sustain and regulate their fate. Extracellular matrix (ECM), paracrine factors or other cells are key niche regulating elements. As the conventional 2D cell culture lacks these elements, it can alter the properties of naïve stem cells. In this work we designed a novel biomimetic microenvironment for cell culture, consisting of magnetic microspheres, prepared with acrylates and acrylic acid copolymers and functionalized with fibronectin or hyaluronic acid as ECM coatings. To characterize cell proliferation and adhesion, porcine mesenchymal stem cells (MSCs) were grown with the different microspheres. The results showed that the 3D environments presented similar proliferation to the 2D culture and that fibronectin allows cell adhesion, while hyaluronic acid hinders it. In the 3D environments, cells reorganize the microspheres to grow in aggregates, highlighting the advantages of microspheres as 3D environments and allowing the cells to adapt the environment to their requirements.

Int J Mol Sci. 2019 Jan 31;20(3). pii: E612. doi: 10.3390/ijms20030612.

Minimally Manipulative Method for the Expansion of Human Bone Marrow Mesenchymal Stem Cells to Treat Osseous Defects.

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Abstract

Lack of standardization of clinically compliant culture protocols of mesenchymal stem cells for re-implantation in humans have hindered clinical progress in the field of tissue regeneration to repair maxillofacial and orthopedic defects. The goal of this study was to establish a clinically relevant osteogenic protocol for collection and expansion of autologous stem cells to be used at Marshall University for re-implantation and repair of maxillofacial and orthopedic conditions. Human bone marrow (hBM) samples were collected from patients undergoing intramedullary nail fixation for closed femoral fractures. hBM mesenchymal cells were expanded by growing them first in Petri dishes for two weeks, followed by a week of culture using Perfecta 3D Hanging Drop Plates®. Various scaffold materials were tested and analyzed for cellular integration, vitality, and differentiation capacity of harvested hBM-MSCs including: 60/40 blend of hydroxyapatite biomatrix; Acellular bone composite discs; Allowash®, cancellous bone cubes; PLGA (poly lactic-co-glycolic acid); and Woven chitin derived fiber. We found that the 3D spheroid culture allowed production of hBM mesenchymal cells that

retained osteoblast differentiation capacity over a monolayer culture of hBM-MSCs without the need to use chemical or hormonal modulation. We also observed that hydroxyapatite and Allowash cancellous bone scaffolds allowed better cell integration and viability properties as compared to other materials tested in this study. In conclusion, the multimodal culture methodology we developed creates actively differentiating stem-cell spheroids that can then be readily utilized in clinical practices to improve the regeneration of tissues of the head and the body.

Aging Dis. 2019 Feb 1;10(1):174-189. doi: 10.14336/AD.2017.1213. eCollection 2019 Feb.

Epigenetic Regulation of Bone Marrow Stem Cell Aging: Revealing Epigenetic Signatures associated with Hematopoietic and Mesenchymal Stem Cell Aging.

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Abstract

In this review we explore the importance of epigenetics as a contributing factor for aging adult stem cells. We summarize the latest findings of epigenetic factors deregulated as adult stem cells age and the consequence on stem cell self-renewal and differentiation, with a focus on adult stem cells in the bone marrow. With the latest whole genome bisulphite sequencing and chromatin immunoprecipitations we are able to decipher an emerging pattern common for adult stem cells in the bone marrow niche and how this might correlate to epigenetic enzymes deregulated during aging. We begin by briefly discussing the initial observations in yeast, drosophila and Caenorhabditis elegans (*C. elegans*) that led to the breakthrough research that identified the role of epigenetic changes associated with lifespan and aging. We then focus on adult stem cells, specifically in the bone marrow, which lends strong support for the deregulation of DNA methyltransferases, histone deacetylases, acetylates, methyltransferases and demethylases in aging stem cells, and how their corresponding epigenetic modifications influence gene expression and the aging phenotype. Given the reversible nature of epigenetic modifications we envisage "epi" targeted therapy as a means to reprogram aged stem cells into their younger counterparts.

Biochim Biophys Acta Mol Cell Res. 2019 Jan 28. pii: S0167-4889(18)30228-3. doi: 10.1016/j.bbamcr.2019.01.012. [Epub ahead of print]

Long lasting inhibition of Mdm2-p53 interaction potentiates mesenchymal stem cell differentiation into osteoblasts.

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Abstract

The osteoblast generation from Mesenchymal stem cells (MSCs) is tightly coordinated by transcriptional networks and signalling pathways that control gene expression and protein stability of osteogenic "master transcription factors". Among these pathways, a great attention has been focused

on p53 and its physiological negative regulator, the E3 ligase Murine double minute 2 (Mdm2). Nevertheless, the signalling that regulates Mdm2-p53 axis in osteoblasts remain to be elucidated, also considering that Mdm2 possesses numerous p53-independent activities and interacts with additional proteins. Herein, the effects of Mdm2 modulation on MSC differentiation were examined by the use of short- and long-lasting inhibitors of the Mdm2-p53 complex. The long-lasting Mdm2-p53 dissociation was demonstrated to enhance the MSC differentiation into osteoblasts. The increase of Mdm2 levels promoted its association to G protein-coupled receptors kinase (GRK) 2, one of the most relevant kinases involved in the desensitization of G protein-coupled receptors (GPCRs). In turn, the long-lasting Mdm2-p53 dissociation decreased GRK2 levels and favoured the functionality of A_{2B} Adenosine Receptors (A_{2B}ARs), a GPCR dictating MSC fate. EB148 facilitated cAMP accumulation, and mediated a sustained activation of extracellular signal-regulated kinases (ERKs) and cAMP response element-binding protein (CREB). Such pro-osteogenic effects were not detectable by using the reversible Mdm2-p53 complex inhibitor, suggesting the time course of Mdm2-p53 dissociation may impact on intracellular proteins involved in cell differentiation fate. These results suggest that the long-lasting Mdm2 binding plays a key role in the mobilization of intracellular proteins that regulate the final biological outcome of MSCs.

Regen Med. 2019 Jan 31. doi: 10.2217/rme-2018-0137. [Epub ahead of print]

An insight into the whole transcriptome profile of four tissue-specific human mesenchymal stem cells.

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Abstract

AIM:

Variations in the clinical outcomes using mesenchymal stem cells (MSCs) treatments exist, reflecting different origins and niches. To date, there is no consensus on the best source of MSCs most suitable to treat a specific disease.

METHODS:

Total transcriptome analysis of human MSCs was performed. MSCs were isolated from two adult sources bone marrow, adipose tissue and two perinatal sources umbilical cord and placenta.

RESULTS:

Each MSCs type possessed a unique expression pattern that reflects an advantage in terms of their potential therapeutic use. Advantages in immune modulation, neurogenesis and other aspects were found.

DISCUSSION:

This study is a milestone for evidence-based choice of the type of MSCs used in the treatment of diseases.

Am J Stem Cells. 2018 Dec 1;7(5):114-120. eCollection 2018.

Impact of donor characteristics on the quality of bone marrow as a source of mesenchymal stromal cells.

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Abstract

In recent years, the therapeutic use of mesenchymal stromal cells (MSC) has generated a valuable number of scientific studies that delve into their biological characteristics and their potential in regenerative medicine; however, the impact of the clinical characteristics of tissue donors, from which these cells are isolated, on their potential in applied clinical research is not yet clear. The objective of this study was to evaluate the impact of the clinical characteristics of bone marrow donors on the quality of this tissue as a source of MSC for therapeutic use. Human MSC were isolated, characterized and cultured (according to ISCT criteria) from bone marrow samples from volunteer donors (n = 70) attending the Department of Orthopedics and Traumatology of the Hospital Universitario San Ignacio (Bogota, Colombia) for surgery of prosthetic hip replacement that agreed to participate voluntarily in the study. Donor data such as age, gender, weight, smoker and type of anesthesia used during the surgical procedure were recorded, and the impact of these characteristics on the volume of tissue collection, mononuclear cell count and confluence time of cells with fibroblastoid morphology was evaluated. Correlation coefficients between quantitative variables were calculated with Spearman's correlation test, and the association between qualitative and quantitative variables was evaluated with biserial correlation coefficient. A significant correlation was observed between the age of the donors and the time necessary to obtain confluent cells in vitro (r = 0.2489, P = 0.0377); similarly, the correlation between the volume of bone marrow collected and the number of mononuclear cells obtained was significant (r = 0.7101, P = 0.0001). Although a negative correlation tendency was observed between the mononuclear cell count and the confluence time, this was not significant (r = -0.2041, P = 0.0950). No significant associations were observed between gender, smoking status or type of anesthesia and the expansion characteristics of human mesenchymal stromal cells. Bone marrow donor age and the tissue collection volume impact the time of obtaining MSC in vitro and the mononuclear cell count with which the culture starts. These conditions must be considered when the bone marrow is selected as the tissue for obtaining MSC.

Cell Death Dis. 2019 Jan 28;10(2):85. doi: 10.1038/s41419-019-1336-4.

IFNβ enhances mesenchymal stromal (Stem) cells immunomodulatory function through STAT1-3 activation and mTOR-associated promotion of glucose metabolism.

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Abstract

Administration of mesenchymal stem cells (MSC) ameliorate experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), at both clinical and neuropathological levels. The therapeutic properties of MSC in EAE are mainly mediated by the modulation of pathogenic immune response, but other neurotropic effects, including decreased demyelination and axonal loss as well as promotion of tissue repair, play also a role. Properly controlled phase II clinical trials to explore the potential of MSC transplantation as a treatment for MS are underway. Interferon beta (IFNβ) is an approved treatment for relapsing-remitting and secondary progressive MS. Here, we explored the possibility that IFNβ might influence the therapeutic potential of MSC, in view of possible synergistic effects as add-on therapy. IFNβ enhanced the immunomodulatory functions of MSC and induced the expression of secretory leukocyte protease inhibitor (Slpi) and hepatocyte growth factor (Hgf), two soluble mediators involved in immune and regenerative functions of MSC. At molecular level, IFNβ induced a rapid and transient phosphorylation of STAT1 and STAT3, the transcription factors responsible for Slpi and Hgf induction. Concomitantly, IFNβ dynamically affected the activity of mTOR, a key checkpoint in the control of metabolic pathways. Indeed, the impairment of mTOR activity observed early upon exposure to IFNβ, was followed by a long-lasting induction of mTOR signaling, that was associated with an increased glycolytic capacity in MSC. When induced to switch their energetic metabolism towards glycolysis, MSC showed an improved ability to control T-cell proliferation. These results suggest that modifications of MSC energetic metabolism induced by IFNβ may contribute to promote MSC immunomodulatory function and support a role for metabolic pathways in the therapeutic function of MSC. Altogether, these findings support the idea of a combined treatment for MS, in which the immunomodulatory and possibly regenerative activity of MSC could be enhanced by the administration of IFNβ.

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Facilitation of chemotaxis activity of mesenchymal stem cells via stromal cell-derived factor-1 and its receptor may promote ectopic ossification of human spinal ligaments.

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Abstract

Mesenchymal stem cells (MSCs) have been utilized to elucidate the pathogenesis of numerous diseases. Our recent study showed that MSCs may conduce to the ossification of spinal ligaments. Stromal cell-derived factor-1 (SDF-1) and CXC chemokine receptor 4 (CXCR4) regulate MSC migration. Moreover, their expression is elevated in sites of damage and remodeling in pathological states. We explored the possible role of the SDF-1/CXCR4 axis in chemotactic behavior of MSCs in ossification of spinal ligaments. Specimens of thoracic vertebra ossified ligamentum flavum (OLF) and non-OLF plaques were received from patients whom we had performed spine surgery. Paraffin-

embedded tissue sections were prepared for immunohistochemical staining. Cultured MSCs from the ligamentum flavum were prepared for in vitro analyses. We observed SDF-1 and CXCR4 localization immunohistochemically in the perivascular area and collagenous matrix of ligaments and in chondrocytes near the ossification front of OLF. And then, immunohistochemical staining showed a close relationship between MSCs and the SDF-1/CXCR4. In the in vitro analyses, expression of the SDF-1/CXCR4 and the migratory capacity of MSCs in OLF were remarkably higher compared with non-OLF. Furthermore, the migration of MSCs was up-regulated by SDF-1 and down-regulated by treatment with AMD3100, a specific antagonist for CXCR4. All in vitro test data showed a significant difference in MSCs from OLF compared with non-OLF. Our results reveal that the SDF-1/CXCR4 axis may contribute to an MSC-mediated increase in the ossification process, indicating that SDF-1/CXCR4 axis may become a potential target for a novel therapeutic strategy for ossification of spinal ligaments

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Exosomes isolation and identification from equine mesenchymal stem cells.

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Abstract

BACKGROUND:

Mesenchymal stem cells are used for different therapeutic approaches, e.g. for osteoarthritis, lesions of the tendon as well as for bone defects. Current research on the mechanism of stem cells on the repair of damaged tissue suggest an important role of a cell-to-cell communication through secreted extracellular vesicles, mainly represented by exosomes. To enhance the scarce knowledge on the functional role of exosomes we compared as a first step different techniques to isolate and identify exosomes from the supernatant of equine adipose derived mesenchymal stem cells for further characterization and usage in functional assays.

RESULTS:

It was possible to obtain exosomes secreted from equine adipose derived mesenchymal stem cells with three common techniques: a stepwise ultracentrifugation at 100.000 g, an ultrafiltration with 3 kDa exclusion membranes and a charge-based precipitation method. The mean sizes and amounts of exosomes isolated with the different techniques were measured by the nanoparticle tracking analysis. The diameter ranged between 116.2 nm (ultracentrifugation), 453.1 nm (precipitation) and 178.7 nm (ultrafiltration), the counts of particles / ml ranged between 9.6×10^8 (ultracentrifugation), 2.02×10^9 (precipitation) and 52.5×10^9 (ultrafiltration). Relevant marker for exosomes, tetraspanins CD9, CD63 and CD81 were detectable by immunofluorescence staining of the investigated exosomes secreting mesenchymal stem cells. In addition, transmission electron microscopy and immunogold labeling with CD9 and CD90 was performed to display the morphological shape of exosomes and existence of marker relevant for exosomes (CD9) and mesenchymal stem cells (CD90). Western blot

analysis of CD9 and CD90 of exosomes ensured the specificity of the rare available respectively cross reacting antibodies against equine antigens.

CONCLUSION:

Exosomes generated by equine mesenchymal stem cells can be obtained by ultrafiltration and ultracentrifugation in an equal quality for in vitro experiments. Especially for later therapeutic usage we recommend ultrafiltration due to a higher concentration without aggregation of extracellular vesicles in comparison to exosomes obtained by ultracentrifugation.

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Determinants of stem cell lineage differentiation toward chondrogenesis versus adipogenesis.

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Abstract

Adult stem cells, also termed as somatic stem cells, are undifferentiated cells, detected among differentiated cells in a tissue or an organ. Adult stem cells can differentiate toward lineage specific cell types of the tissue or organ in which they reside. They also have the ability to differentiate into mature cells of mesenchymal tissues, such as cartilage, fat and bone. Despite the fact that the balance has been comprehensively scrutinized between adipogenesis and osteogenesis and between chondrogenesis and osteogenesis, few reviews discuss the relationship between chondrogenesis and adipogenesis. In this review, the developmental and transcriptional crosstalk of chondrogenic and adipogenic lineages are briefly explored, followed by elucidation of signaling pathways and external factors guiding lineage determination between chondrogenic and adipogenic differentiation. An in-depth understanding of overlap and discrepancy between these two mesenchymal tissues in lineage differentiation would benefit regeneration of high-quality cartilage tissues and adipose tissues for clinical applications.