ML 02-19 (15/01/2019)

J Transl Med. 2019 Jan 5;17(1):10. doi: 10.1186/s12967-018-1750-x.

Isolation of clinically relevant concentrations of bone marrow mesenchymal stem cells without centrifugation.

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Author information

Abstract

BACKGROUND:

This study examined the quality of bone marrow aspirates extracted using a novel, FDA cleared method to optimally target cells from the inner cortical iliac bone surface without the need for centrifugation. This method employs small draws from a single puncture that promote only lateral flow from multiple sites (SSLM method). The study utilized the Marrow Cellutions bone marrow aspiration system (MC system) which is based on the SSLM method and compared the MC system directly to bone marrow concentrates (BMAC) generated by centrifugation of aspirates harvested with a standard aspiration needle.

METHODS:

Three direct comparisons were conducted evaluating the SSLM draws and BMACs derived from the same patient from contralateral iliac crests. The levels of TNCs/mL, CD34+ cells/mL, CD117+ cells/mL, and CFU-f/mL were compared between the various bone marrow preparations. The cellular content of a series of SSLM draws was also analyzed to determine the total nucleated cell (TNC) count and the concentration of mesenchymal stem/progenitor cells as measured by colony forming unit fibroblasts (CFU-f).

RESULTS:

In direct comparisons with BMAC systems, SSLM draws yielded significantly higher CFU-f concentrations and comparable concentrations of CD34+ and CD117+ cells. In addition, the average quantity of TNCs/mL in a series of 30 patients utilizing the SSLM draw was $35.2 \times 10^6 \pm 17.1 \times 10^6$ and the average number of CFU-f/mL was 2885 ± 1716. There were small but significant correlations between the TNCs/mL and the CFU-fs/mL using the SSLM method as well as between the age of the patient and the CFU-fs/mL.

CONCLUSIONS:

The MC Device, using the SSLM draw technique, produced concentrations of CFU-fs, CD34+ cells and CD117+ cells that were comparable or greater to BMACs derived from the same patient. Given the rapid speed and simplicity of the MC Device, we believe this novel system possesses significant practical advantages to other currently available centrifugation based systems.

Curr Protoc Stem Cell Biol. 2019 Jan 9:e76. doi: 10.1002/cpsc.76. [Epub ahead of print]

Isolation and Flow Cytometry Characterization of Extracellular-Vesicle Subpopulations Derived from Human Mesenchymal Stromal Cells.

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Abstract

This unit describes protocols for isolating subpopulations of extracellular vesicles (EVs) purified from human adipose tissue-derived mesenchymal stromal cells by density gradient centrifugation and for characterizing them by flow cytometry (FCM). Determining the optimal strategy for isolating EVs is a critical step toward retrieving the maximal amount while ensuring the recovery of different vesicular subtypes. The first protocol details density gradient centrifugation to isolate both exosomes and microvesicles. In the second protocol, characterization of EV subpopulations by FCM is depicted, taking advantage of non-conventional modalities, in accordance with the latest technical indications. The procedures described here can be easily reproduced and can be employed regardless of the cell type used to obtain EVs

Front Bioeng Biotechnol. 2018 Dec 21;6:203. doi: 10.3389/fbioe.2018.00203. eCollection 2018.

Platelet Lysate Activates Human Subcutaneous Adipose Tissue Cells by Promoting Cell Proliferation and Their Paracrine Activity Toward Epidermal Keratinocytes.

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Abstract

Skin chronic wounds are non-healing ulcerative defects, which arise in association with a morbidity state, such as diabetes and vascular insufficiency or as the consequence of systemic factors including advanced age. Platelet Rich Plasma, a platelet-rich blood fraction, can significantly improve the healing of human skin chronic ulcers. Given that the subcutaneous adipose tissue is located beneath the skin and plays a role in the skin homeostasis, in this study, we investigated the *in vitro* response of human subcutaneous adipose tissue cells to platelet content in a model mimicking *in vitro* the *in situ* milieu of a deep skin injury. Considering that, at the wound site, plasma turn to serum, platelets are activated and inflammation occurs, human adipose-derived stromal cells (hASC) were cultured with Human Serum (HS) supplemented or not with Platelet Lysate (PL) and/or IL-1α. We observed that HS sustained hASC proliferation more efficiently than FBS and induced a spontaneous adipogenic differentiation in the cells. PL added to HS enhanced hASC proliferation, regardless the presence of IL-1α. In the presence of PL, hASC progressively lessened the adipogenic phenotype, possibly because the proliferation of less committed cells was induced. However, these cells resumed adipogenesis in permissive conditions. Accordingly, PL induced in quiescent cells activation of the proliferation-related pathways

ERK, Akt, and STAT-3 and expression of Cyclin D1. Moreover, PL induced an early and transient increase of the pro-inflammatory response triggered by IL-1α, by inducing COX-2 expression and secretion of a large amount of PGE_2, IL-6, and IL-8. Media conditioned by PL-stimulated hASC exerted a chemotactic activity on human keratinocytes and favored the healing of an *in vitro* scratch wound. In order to bridge the gap between *in vitro* results and possible *in vivo* events, the stimuli were also tested in *ex vivo* cultures of *in toto* human adipose tissue biopsies (hAT). PL induced cell proliferation in hAT and outgrowth of committed progenitor cells able to differentiate in permissive conditions. In conclusion, we report that the adipose tissue responds to the wound microenvironment by activating the proliferation of adipose tissue progenitor cells and promoting the release of factors favoring wound healing.

Stem Cell Res Ther. 2019 Jan 11;10(1):13. doi: 10.1186/s13287-018-1114-8.

Melatonin plays critical role in mesenchymal stem cell-based regenerative medicine in vitro and in vivo.

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Abstract

Although stem cells have emerged as promising sources for regenerative medicine, there are many potential safety hazards for their clinical application, including tumorigenicity, an availability shortage, senescence, and sensitivity to toxic environments. Mesenchymal stem cells (MSCs) have various advantages compared to other stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs); thus, MSCs have been intensely investigated in recent studies. However, they are placed in a harsh environment after isolation and transplantation, and the adverse microenvironment substantially reduces the viability and therapeutic effects of MSCs. Intriguingly, melatonin (MT), which is primarily secreted by the pineal organ, has been found to influence the fate of MSCs during various physiological and pathological processes. In this review, we will focus on the recent progress made regarding the influence of MT on stem cell biology and its implications for regenerative medicine. In addition, several biomaterials have been proven to significantly improve the protective effects of MT on MSCs by controlling the release of MT. Collectively, MT will be a promising agent for enhancing MSC activities and the regenerative capacity via the regulation of reactive oxygen species (ROS) generation and the release of immune factors in regenerative medicine.

J Cell Physiol. 2019 Jan 11. doi: 10.1002/jcp.28099. [Epub ahead of print]

How is mechanobiology involved in mesenchymal stem cell differentiation toward the osteoblastic or adipogenic fate?

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Abstract

Mechanobiology plays a major role in transducing physical cues from the dynamic cellular environment into biochemical modifications that promote cell-specific differentiation paths. Mesenchymal stem cells in the bone marrow or in other mesenchymal tissues will differentiate according to the expression of transcription factors (TFs) that govern their lineage commitment. The favoring of either osteogenic or adipogenic differentiation relies on TF expression as well as mechanical properties of the cells' niche that are translated into the activation of certain signaling pathways. Physical factors can induce significant shifts in bipotential lineage commitment between osteogenesis and adipogenesis. The stiffness of the extracellular matrix (ECM) surrounding a cell, varying greatly from rigid environments close to the bone surface to softer regions in the bone marrow, can influence the path of differentiation. Additionally, mechanical loading through exercise appears to favor osteogenesis whereas disuse conditions seem to promote adipogenesis.

Stem Cell Res Ther. 2019 Jan 10;10(1):10. doi: 10.1186/s13287-018-1103-y.

Cell number in mesenchymal stem cell aggregates dictates cell stiffness and chondrogenesis.

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Abstract

BACKGROUND:

Although mesenchymal stem/stromal cell (MSC) chondrogenic differentiation has been thoroughly investigated, the rudiments for enhancing chondrogenesis have remained largely dependent on external cues. Focus to date has been on extrinsic variables such as soluble signals, culture conditions (bioreactors), and mechanical stimulation. However, the role of intrinsic mechanisms of MSC programming-based mechanobiology remains to be explored. Since aggregation of MSCs, a prerequisite for chondrogenesis, generates tension within the cell agglomerate, we inquired if the initial number of cells forming the aggregate (aggregate cell number (ACN)) can impact chondrogenesis.

METHODS:

Aggregates of varying ACN were formed using well-established centrifugation approach. Progression of chondrogenic differentiation in the aggregates was assessed over 3 weeks in presence and absence of transforming growth factor-beta 1 (TGF-β1). Mechanical properties of the cells were characterized using high-throughput real-time deformability cytometry (RT-DC), and gene expression was analyzed using Affymetrix gene array. Expression of molecular markers linked to chondrogenesis was assessed using western blot and immunofluorescence.

RESULTS:

Reducing ACN from 500 k to 70 k lead to activation and acceleration of the chondrogenic differentiation, independent of soluble chondro-inductive factors, which involves changes to β-catenin-dependent TCF/LEF transcriptional activity and expression of anti-apoptotic protein survivin. RT-DC analysis revealed that stiffness and size of cells within aggregates are modulated by ACN. A direct correlation

between progression of chondrogenesis and emergence of stiffer cell phenotype was found. Affymetrix gene array analysis revealed a downregulation of genes associated with lipid synthesis and regulation, which could account for observed changes in cell stiffness. Immunofluorescence and western blot analysis revealed that increasing ACN upregulates the expression of lipid raft protein caveolin-1, a β-catenin binding partner, and downregulates the expression of N-cadherin. As a demonstration of the relevance of these findings in MSC-based strategies for skeletal repair, it is shown that implanting aggregates within collagenous matrix not only decreases the necessity for high cell numbers but also leads to marked improvement in the quality of the deposited tissue.

CONCLUSIONS:

This study presents a simple and donor-independent strategy to enhance the efficiency of MSC chondrogenic differentiation and identifies changes in cell mechanics coincident with MSC chondrogenesis with potential translational applications.

J Orthop Res. 2019 Jan 9. doi: 10.1002/jor.24215. [Epub ahead of print]

2-step stem cell therapy improves bone regeneration compared to concentrated bone marrow therapy.

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Abstract

Adult stem cells are a promising tool to positively influence bone regeneration. Concentrated bone marrow therapy entails isolating osteoprogenitor cells during surgery with, however, only low cells yield. Two step stem cell therapy requires an additional harvesting procedure but generates high numbers of progenitor cells that facilitate osteogenic pre-differentiation. To further improve bone regeneration, stem cell therapy can be combined with growth factors from platelet rich plasma (PRP) or its lysate (PL) to potentially fostering vascularization. The aim of this study was to investigate the effects of bone marrow concentrate (BMC), osteogenic pre-differentiation of mesenchymal stromal cells (MSCs) and PL on bone regeneration and vascularization. Bone marrow from 4 different healthy human donors was used for either generation of BMC or for isolation of MSCs. Seventy-two mice were randomized to 6 groups (Control, PL, BMC, BMC + PL, pre-differentiated MSCs, pre-differentiated MSCs + PL). The influence of PL, BMC and pre-differentiated MSCs was investigated systematically in a 2 mm femoral bone defect model. After a 6-week follow-up, the pre-differentiated MSCs + PL group showed the highest bone volume, highest grade of histological defect healing and highest number of bridged defects with measurable biomechanical stiffness. Using expanded and osteogenically pre-differentiated MSCs for treatment of a critical-size bone defect was favorable with regards to bone regeneration compared to treatment with cells from BMC. The addition of PL alone had no significant influence; therefore the role of PL for bone regeneration remains unclear.

J Tissue Eng. 2018 Dec 25;9:2041731418810093. doi: 10.1177/2041731418810093. eCollection 2018 Jan-Dec.

Mesenchymal stem cell-derived extracellular vesicles may promote breast cancer cell dormancy.

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Abstract

Disseminated breast cancer cells have the capacity to metastasise to the bone marrow and reside in a dormant state within the mesenchymal stem cell niche. Research has focussed on paracrine signalling factors, such as soluble proteins, within the microenvironment. However, it is now clear extracellular vesicles secreted by resident mesenchymal stem cells into this microenvironment also play a key role in the initiation of dormancy. Dormancy encourages reduced cell proliferation and migration, while upregulating cell adhesion, thus retaining the cancer cells within the bone marrow microenvironment. Here, MCF7 breast cancer cells were treated with mesenchymal stem cell-derived extracellular vesicles, resulting in reduced migration in two-dimensional and three-dimensional culture, with reduced cell proliferation and enhanced adhesion, collectively supporting cancer cell dormancy.

J Clin Periodontol. 2019 Jan 9. doi: 10.1111/jcpe.13053. [Epub ahead of print]

Mesenchymal Stem Cells and Biologic Factors leading to Bone Formation.

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Author information

Abstract

BACKGROUND:

Physiological bone formation and bone regeneration occurring during bone repair can be considered distinct but similar processes. Mesenchymal stem cells and associated biologic factors are crucial to both bone formation and bone regeneration.

AIM:

To perform a narrative review of the current literature regarding the role of mesenchymal stem cells and biologic factors in bone formation with the aim of discussing the clinical relevance of in vitro and in vivo animal studies.

METHODS:

The literature was searched for studies on mesenchymal stem cells and biologic factors associated with the formation of bone in the mandible and maxilla. The search specifically targeted studies on key aspects of how stem cells and biologic factors are important in bone formation and how this might be relevant to bone regeneration. The results are summarized in a narrative review format.

RESULTS:

Different types of mesenchymal stem cells and many biologic factors are associated with bone formation in the maxilla and mandible.

CONCLUSION:

Bone formation and regeneration involve very complex and highly regulated cellular and molecular processes. By studying these processes new clinical opportunities will arise for therapeutic bone regenerative treatments.