ML 35-19 (28/11/2019)

Stem Cells Transl Med

2019 Nov 23[Online ahead of print]

Single-cell High Content Imaging Parameters Predict Functional Phenotype of Cultured Human Bone Marrow Stromal Stem Cells

Justyna M Kowal, Hagen Schmal ... Moustapha Kassemexpand

- PMID: 31758755

- DOI: 10.1002/sctm.19-0171

Abstract

Cultured human bone marrow stromal (mesenchymal) stem cells (hBM-MSCs) are heterogenous cell populations exhibiting variable biological properties. Quantitative high-content imaging technology allows identification of morphological markers at a single cell resolution that are determinant for cellular functions. We determined the morphological characteristics of cultured primary hBM-MSCs and examined their predictive value for hBM-MSC functionality. BM-MSCs were isolated from 56 donors and characterized for their proliferative and differentiation potential. We correlated these data with cellular and nuclear morphological features determined by Operetta; a high-content imaging system. Cell area, cell- and nucleus geometry of cultured hBM-MSCs exhibited significant correlation with expression of hBM-MSC membrane markers: ALP, CD146, CD271. Proliferation capacity correlated negatively with cell and nucleus area and positively with cytoskeleton texture features. In addition, in vitro differentiation to osteoblasts as well as in vivo heterotopic bone formation was associated with decreased ratio of nucleus width to length. Multivariable analysis applying a stability selection procedure identified nuclear geometry and texture as predictors for hBM-MSCs differentiation potential to osteoblasts or adipocytes. Our data demonstrate that by employing a limited number of cell morphological characteristics, it is possible to predict the functional phenotype of cultured hBM-MSCs and thus can be used as a screening test for "quality" of hBM-MSCs prior their use in clinical protocols.

Biomaterials

, 119571
2019 Oct 24[Online ahead of print]

Exosomes Influence the Behavior of Human Mesenchymal Stem Cells on Titanium Surfaces

Xiaoqin Wang, Furqan A Shah ... Peter Thomsenexpand

- PMID: 31753474

- DOI: 10.1016/j.biomaterials.2019.119571

Abstract

Mesenchymal stem cells (MSCs) have important roles during osseointegration. This study determined (i) if MSC-derived extracellular vesicles (EVs)/exosomes can be immobilized on titanium (Ti) surfaces and influence the behavior of MSCs, (ii) if the response is differentially affected by EVs from expanded vs differentiated MSCs and (iii) if the EV protein cargos predict the functional features of the exosomes. EVs secreted by human adipose-derived MSCs were isolated by ultracentrifugation and analyzed using nanoparticle tracking analysis, Western blotting and relative quantitative mass spectrometry. Fluorescence microscopy, scanning electron microscopy, cell counting assay and quantitative polymerase chain reaction were used to analyze MSC adhesion, proliferation and differentiation. Exosome immobilization on Ti promoted MSC adhesion and spreading after 24 h and proliferation after 3 and 6 days, irrespective of whether the exosomes were obtained from expansion or differentiation conditions. Immobilized exosomes upregulated stromal cell-derived factor (SDF-1α) gene expression. Cell adhesion molecules and signaling molecules were abundant in the exosomal proteome. The predicted functions of the equally-abundant proteins in both exosome types were in line with the observed biological effects mediated by the exosomes. Thus, exosomes derived from MSCs and immobilized on Ti surfaces interact with MSCs and rapidly promote MSC adhesion and proliferation. These findings provide a novel route for modification of titanium implant surfaces.

Stem Cell Res Ther

, 10 (1), 338
2019 Nov 21

Surgical Vacuum Filter-Derived Stromal Cells Are Superior in Proliferation to Human Bone Marrow Aspirate

Katharina Henze, Monika Herten ... Marcus Jägerexpand

- PMID: 31753037

- DOI: 10.1186/s13287-019-1461-0

Abstract

Background: During joint replacement, surgical vacuum suction guarantees a sufficient overview on the situs. We assume high concentrations of mesenchymal stromal cells (MSCs) on surgical vacuum filters. We compared the in vitro proliferative and differentiation potency of cells from the following: (i) bone marrow (BM), (ii) cancellous bone (CB), (iii) vacuum filter (VF), and (iv) cell saver filtrate reservoir (SF) in 32 patients undergoing elective total hip replacement.

Methods: Mononuclear cells (MNC) were isolated, and cell proliferation and colony-forming units (CFU) were measured. Adherent cells were characterized by flow cytometry for MSC surface markers. Cells were incubated with osteogenic, adipogenic, and chondrogenic stimuli. Cells were cytochemically stained and osteoblastic expression (RUNX-2, ALP, and BMP-2) investigated via qPCR.

Results: Dependent on the source, initial MNC amount as well as CFU number was significantly different whereas generation time did not vary significantly. CFU numbers from VF were superior to those from SR, BM, and CB. The resulting amount of MSC from the respective source was highest in the vacuum filter followed by reservoir, aspirate, and cancellous bone. Cells from all groups could be differentiated into the three mesenchymal lines demonstrating their stemness nature. However, gene expression of osteoblastic markers did not differ significantly between the groups.

Conclusion: We conclude that surgical vacuum filters are able to concentrate tissue with relevant amounts of MSCs. A new potent source of autologous regeneration material with

clinical significance is identified. Further clinical studies have to elucidate the regenerative potential of this material in an autologous setting.

Int J Mol Sci

, 20 (22)
2019 Nov 18

Calcium Polyphosphate Nanoparticles Act as an Effective Inorganic Phosphate Source During Osteogenic Differentiation of Human Mesenchymal Stem Cells

Luan Phelipe Hatt, Keith Thompson ... Angela Rita Armientoexpand

- PMID: 31752206

- DOI: 10.3390/ijms20225801

Abstract

The ability of bone-marrow-derived mesenchymal stem/stromal cells (BM-MSCs) to differentiate into osteoblasts makes them the ideal candidate for cell-based therapies targeting bone-diseases. Polyphosphate (polyP) is increasingly being studied as a potential inorganic source of phosphate for extracellular matrix mineralisation. The aim of this study is to investigate whether polyP can effectively be used as a phosphate source during the in vitro osteogenic differentiation of human BM-MSCs. Human BM-MSCs are cultivated under osteogenic conditions for 28 days with phosphate provided in the form of organic β-glycerolphosphate (BGP) or calcium-polyP nanoparticles (polyP-NP). Mineralisation is demonstrated using Alizarin red staining, cellular ATP content, and free phosphate levels are measured in both the cells and the medium. The effects of BGP or polyP-NP on alkaline phosphatase (ALP) activity and gene expression of a range of osteogenic-related markers are also assessed. PolyP-NP supplementation displays comparable effects to the classical BGP-containing osteogenic media in terms of mineralisation, ALP activity and expression of osteogenesis-associated genes. This study shows that polyP-NP act as an effective source of phosphate during mineralisation of BM-MSC. These results open new possibilities with BM-MSC-based approaches for bone repair to be achieved through doping of conventional biomaterials with polyP-NP.

J Theor Biol

, 110086
2019 Nov 18[Online ahead of print]

Cells Adapt Their Shapes With Their Experienced Stiffness: A Geometrical Approach to Differentiation

Amir Hossein Hajiexpand

- PMID: 31751576

- DOI: 10.1016/j.jtbi.2019.110086

Abstract

The elasticity-directed differentiation of mesenchymal stem cells has been widely studied since mid 2000s. Over nearly linear-elastic materials the differentiation of the stem cells are shown to be related to the Young's modulus of the substrate. While it is found that constraining the stem cells to some pre-patterned shape and size affects their differentiation, it has been also well recognized that the cell morphology intimately relates to its type and functionality such that the cell morphology has been accepted as an indicator for the differentiation path. This paper conjectures the importance of geometry in differentiation and claims that the elasticity indicator for differentiation is indeed "the stiffness" which contains not only the elasticity coefficient but also the geometrical information. An elasticity model is derived for a singular cell with different shapes over a thick substrate, which almost resembles the condition for most of the tests with sparse distribution of the cells. Analysis of principle shapes such as square, rectangle, and hexagon (with and without dendrites) suggest that the larger the aspect ratio i.e. the further the shape to the roundness, the larger the substrate stiffness experienced by the cells. By moving towards a more round shape such as a hexagon with or without dendrites the substrate stiffness falls off rapidly. Then by including the stiffness of the cell body itself we arrive at a more important finding; the cells at free culture condition prefer the shape which best equalizes their experienced stiffness of the substrate and that of their own body. The body-to-substrate stiffness ratio, hence, explains why a slender rectangle is the preferred shape at myogenic differentiation range and a hexagon with dendrites is the preferred one at neurogenic range.

PLoS One

, 14 (11), e0225472
2019 Nov 21 eCollection 2019

Exosomes From Conditioned Media of Bone Marrow-Derived Mesenchymal Stem Cells Promote Bone Regeneration by Enhancing Angiogenesis

Ryoko Takeuchi, Wataru Katagiri ... Tadaharu Kobayashiexpand

- PMID: 31751396

- DOI: 10.1371/journal.pone.0225472

Abstract

Growth factors in serum-free conditioned media from human bone marrow-derived mesenchymal stem cells (MSC-CM) are known to be effective in bone regeneration. However, the secretomes in MSC-CM that act as active ingredients for bone regeneration, as well as their mechanisms, remains unclear. Exosomes, components of MSC-CM, provide the recipient cells with genetic information and enhance the recipient cellular paracrine stimulation, which contributes to tissue regeneration. We hypothesized that MSC-CM-derived exosomes (MSC-Exo) promoted bone regeneration, and that angiogenesis was a key step. Here, we prepared an MSC-Exo group, MSC-CM group, and Exo-antiVEGF group (MSC-Exo with angiogenesis inhibitor), and examined the osteogenic and angiogenic potential in MSCs. Furthermore, we used a rat model of calvaria bone defect and implanted each sample to evaluate bone formation weekly, until week 4 after treatment. Results showed that MSC-Exo enhanced cellular migration and osteogenic and angiogenic gene expression in MSCs compared to that in other groups. In vivo, early bone formation by MSC-Exo was also confirmed. Two weeks after implantation, the newly formed bone area was 31.5 ± 6.5% in the MSC-Exo group while those in the control and Exo-antiVEGF groups were 15.4 ± 4.4% and 8.7 ± 1.1%, respectively. Four weeks after implantation, differences in the area between the MSC-Exo group and the Exo-antiVEGF or control groups were further broadened. Histologically, notable accumulation of osteoblast-like cells and vascular endothelial cells was observed in the MSC-Exo group; however, fewer cells were found in the Exo-antiVEGF and control groups. In conclusion, MSC-Exo

promoted bone regeneration during early stages, as well as enhanced angiogenesis. Considering the tissue regeneration with transplanted cells and their secretomes, this study suggests that exosomes might play an important role, especially in angiogenesis.

Stem Cell Res Ther

, 10 (1), 331
2019 Nov 20

CD51 Distinguishes a Subpopulation of Bone Marrow Mesenchymal Stem Cells With Distinct Migratory Potential: A Novel Cell-Based Strategy to Treat Acute Myocardial Infarction in Mice

Dong-Mei Xie, Yuan-Long Li ... Xiuren Gaoexpand

- PMID: 31747966

- DOI: 10.1186/s13287-019-1439-y

Abstract

Background: Experimental and clinical trials have demonstrated the efficiency of bone marrow-derived mesenchymal stromal/stem cells (bMSCs) in the treatment of myocardial infarction. However, after intravenous injection, the ineffective migration of engrafted bMSCs to the hearts remains an obstacle, which has an undesirable impact on the efficiency of cell-based therapy. Therefore, we attempted to identify a marker that could distinguish a subpopulation of bMSCs with a promising migratory capacity.

Methods: Here, CD51-negative and CD51-positive cells were isolated by flow cytometry from $Ter119^{-}CD45^{-}CD31^{-}$bMSCs and cultured in specifically modified medium. The proliferation ability of the cells was evaluated by 5-ethynyl-2'-deoxyuridine (EdU) staining or continuously monitored during culture, and the differentiation potential was assessed by culturing the cells in the appropriate conditioned media. Wound healing assays, transwell assays and quantitative polymerase chain reaction (qPCR) were used to measure the migratory ability. The mice were subjected to a sham operation or myocardial infarction (MI) by permanently occluding the coronary artery, and green fluorescent protein (GFP)-labelled cells were transplanted into the mice via intravenous infusion

immediately after MI. Heart function was measured by echocardiography; infarct myocardium tissues were detected by triphenyl tetrazolium chloride (TTC) staining. Additionally, immunofluorescence staining was used to verify the characteristics of $CD51^+$bMSCs and inflammatory responses in vivo. Statistical comparisons were performed using a two-tailed Student's t test.

Results: In this study, the isolated $CD51^-$bMSCs and $CD51^+$bMSCs, especially the $CD51^+$ cells, presented a favourable proliferative capacity and could differentiate into adipocytes, osteocytes and chondrocytes in vitro. After the cells were transplanted into the MI mice by intravenous injection, the therapeutic efficiency of $CD51^+$bMSCs in improving left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) was better than that of $CD51^-$bMSCs. Compared with $CD51^-$bMSCs, $CD51^+$bMSCs preferentially migrated to and were retained in the infarcted hearts at 48 h and 8 days after intravenous injection. Accordingly, the migratory capacity of $CD51^+$bMSCs exceeded that of $CD51^-$bMSCs in vitro, and the former cells expressed higher levels of chemokine receptors or ligands. Interestingly, the retained $CD51^+$bMSCs retained in the myocardium possessed proliferative potential but only differentiated into endothelial cells, smooth muscle cells, fibroblasts or cardiomyocytes. Transplantation of $CD51^+$bMSCs partially attenuated the inflammatory response in the hearts after MI, while the potential for inflammatory suppression was low in $CD51^-$bMSC-treated mice.

Conclusions: These findings indicated that the CD51-distinguished subpopulation of bMSCs facilitated proliferation and migration both in vitro and in vivo, which provided a novel cell-based strategy to treat acute MI in mice by intravenous injection.

J Biomed Mater Res A

2019 Nov 19[Online ahead of print]

Dose-dependent Cytotoxicity Induced by Pristine Graphene Oxide Nanosheets for Potential Bone Tissue Regeneration

Xiliu Zhang, Changbo Wei ... Dongsheng Yuexpand

- PMID: 31742875

- DOI: 10.1002/jbm.a.36841

Abstract

This study was aimed to investigate the toxic effects of pristine graphene oxide (GO) nanosheets on bone-marrow-derived mesenchymal stem cells (BMSCs), a type of traditional seed cells in tissue regeneration engineering. First, a GO suspension was prepared and characterized by transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), and Raman shifts. Then, rat BMSCs were isolated and characterized. Subsequently, cell proliferation, membrane integrity, cell cycle, cell apoptosis, mitochondrial membrane potential (MMP), and reactive oxygen species (ROS) were measured. In addition, relevant proteins of the mitochondrial apoptotic pathway and autophagy were analyzed. Our results showed that a high concentration of GO inhibited cell viability and membrane integrity, while cell apoptosis and cell-cycle arrest were induced by GO. Further, GO significantly increased ROS generation and MMP loss with an upregulation of Cleaved Caspase-3, LC3-II/I, and Beclin-1 and a downregulation of Bcl-2 and Caspase3. We concluded that the toxic effects of GO on BMSCs occurred in a dose-dependent manner via the mitochondrial apoptotic pathway and autophagy.