Adipose Derived-Mesenchymal Stem Cells Viability and Differentiating Features for Orthopaedic Reparative Applications: Banking of Adipose Tissue.

Roato I¹, Alotto D², Belisario DC¹, Casarin S², Fumagalli M², Cambieri I³, Piana R³, Stella M², Ferracini R³, Castagnoli C².

Author information

Abstract

Osteoarthritis is characterized by loss of articular cartilage also due to reduced chondrogenic activity of mesenchymal stem cells (MSCs) from patients. Adipose tissue is an attractive source of MSCs (ATD-MSCs), representing an effective tool for reparative medicine, particularly for treatment of osteoarthritis, due to their chondrogenic and osteogenic differentiation capability. The treatment of symptomatic knee arthritis with ATD-MSCs proved effective with a single infusion, but multiple infusions could be also more efficacious. Here we studied some crucial aspects of adipose tissue banking procedures, evaluating ATD-MSCs viability, and differentiation capability after cryopreservation, to guarantee the quality of the tissue for multiple infusions. We reported that the presence of local anesthetic during lipoaspiration negatively affects cell viability of cryopreserved adipose tissue and cell growth of ATD-MSCs in culture. We observed that DMSO guarantees a faster growth of ATD-MSCs in culture than trehalose. At last, ATD-MSCs derived from fresh and cryopreserved samples at -80°C and -196°C showed viability and differentiation ability comparable to fresh samples. These data indicate that cryopreservation of adipose tissue at -80°C and -196°C is equivalent and preserves the content of ATD-MSCs in Stromal Vascular Fraction (SVF), guaranteeing the differentiation ability of ATD-MSCs.

Cannabidiol Modulates the Expression of Alzheimer's Disease-Related Genes in Mesenchymal Stem Cells.

Libro R¹, Diomede F², Scionti D³, Piattelli A⁴, Grassi G⁵, Pollastro F⁶, Bramanti P⁷, Mazzon E⁸, Trubiani O⁹.

Author information

Abstract

Mesenchymal stem cells (MSCs) have emerged as a promising tool for the treatment of several neurodegenerative disorders, including Alzheimer's disease (AD). The main neuropathological hallmarks of AD are senile plaques, composed of amyloid beta (Aβ), and neurofibrillary tangles, formed by hyperphosphorylated tau. However, current therapies for AD have shown limited efficacy. In this study, we evaluated whether pre-treatment with cannabidiol (CBD), at 5 μM concentration, modulated the transcriptional profile of MSCs derived from gingiva (GMSCs) in order to improve their therapeutic potential, by performing a transcriptomic analysis by the next-generation sequencing (NGS) platform. By comparing the expression profiles between GMSCs treated with CBD (CBD-GMSCs) and control...
GMSCs (CTR-GMSCs), we found that CBD led to the downregulation of genes linked to AD, including genes coding for the kinases responsible of tau phosphorylation and for the secretases involved in Aβ generation. In parallel, immunocytochemistry analysis has shown that CBD inhibited the expression of GSK3β, a central player in AD pathogenesis, by promoting PI3K/Akt signalling. In order to understand through which receptor CBD exerted these effects, we have performed pre-treatments with receptor antagonists for the cannabinoid receptors (SR141716A and AM630) or for the vanilloid receptor 1 (TRPV1). Here, we have proved that TRPV1 was able to mediate the modulatory effect of CBD on the PI3K/Akt/GSK3β axis. In conclusion, we have found that pre-treatment with CBD prevented the expression of proteins potentially involved in tau phosphorylation and Aβ production in GMSCs. Therefore, we suggested that GMSCs preconditioned with CBD possess a molecular profile that might be more beneficial for the treatment of AD.


Cardiopoietic cell therapy for advanced ischemic heart failure: results at 39 weeks of the prospective, randomized, double blind, sham-controlled CHART-1 clinical trial.


Author information

Abstract

AIMS:
Cardiopoietic cells, produced through cardiogenic conditioning of patients' mesenchymal stem cells, have shown preliminary efficacy. The Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial aimed to validate cardiopoiesis-based biotherapy in a larger heart failure cohort.

METHODS AND RESULTS:
This multinational, randomized, double-blind, sham-controlled study was conducted in 39 hospitals. Patients with symptomatic ischemic heart failure on guideline-directed therapy (n = 484) were screened; n = 348 underwent bone marrow harvest and mesenchymal stem cell expansion. Those achieving > 24 million mesenchymal stem cells (n = 315) were randomized to cardiopoietic cells delivered endomyocardially with a retention-enhanced catheter (n = 157) or sham procedure (n = 158). Procedures were performed as randomized in 271 patients (n = 120 cardiopoietic cells, n = 151 sham). The primary efficacy endpoint was a Finkelstein-Schoenfeld hierarchical composite (all-cause mortality, worsening heart failure, Minnesota Living with Heart Failure Questionnaire score, 6-min walk distance,
left ventricular end-systolic volume, and ejection fraction) at 39 weeks. The primary outcome was neutral (Mann-Whitney estimator 0.54, 95% confidence interval [CI] 0.47-0.61 [value > 0.5 favours cell treatment], P = 0.27). Exploratory analyses suggested a benefit of cell treatment on the primary composite in patients with baseline left ventricular end-diastolic volume 200-370 mL (60% of patients) (Mann-Whitney estimator 0.61, 95% CI 0.52-0.70, P = 0.015). No difference was observed in serious adverse events. One (0.9%) cardiopoietic cell patient and 9 (5.4%) sham patients experienced aborted or sudden cardiac death.

**CONCLUSION:**
The primary endpoint was neutral, with safety demonstrated across the cohort. Further evaluation of cardiopoietic cell therapy in patients with elevated end-diastolic volume is warranted.

**Proteasome activation enhances stemness and lifespan of human mesenchymal stem cells.**
Kapetanou M¹, Chondrogianni N², Petrakis S³, Koliakos G⁴, Gonos ES⁵.

**Author information**

**Abstract**
The age-associated decline of adult stem cell function contributes to the physiological failure of homeostasis during aging. The proteasome plays a key role in the maintenance of proteostasis and its failure is associated with various biological phenomena including senescence and aging. Although stem cell biology has attracted intense attention, the role of proteasome in stemness and its age-dependent deterioration remains largely unclear. By employing both Wharton's-Jelly- and Adipose-derived human adult mesenchymal stem cells (hMSCs), we reveal a significant age-related decline in proteasome content and peptidase activities, accompanied by alterations of proteasomal complexes. Additionally, we show that senescence and the concomitant failure of proteostasis negatively affects stemness. Remarkably, the loss of proliferative capacity and stemness of hMSCs can be counteracted through proteasome activation. At the mechanistic level, we demonstrate for the first time that Oct4 binds at the promoter region of β2 and β5 proteasome subunits and thus possibly regulates their expression. A firm understanding of the mechanisms regulating proteostasis in stem cells will pave the way to innovative stem cell-based interventions to improve healthspan and lifespan.

**Mesenchymal stem cells cultured on magnetic nanowire substrates.**
Perez JE¹, Ravasi T, Kosel J.

**Author information**

**Abstract**
Stem cells have been shown to respond to extracellular mechanical stimuli by regulating their fate through the activation of specific signaling pathways. In this work, an array of iron nanowires (NWs)
aligned perpendicularly to the surface was fabricated by pulsed electrodeposition in porous alumina templates followed by a partial removal of the alumina to reveal 2-3 μm of the NWs. This resulted in alumina substrates with densely arranged NWs of 33 nm in diameter separated by 100 nm. The substrates were characterized by scanning electron microscopy (SEM) energy dispersive x-ray analysis and vibrating sample magnetometer. The NW array was then used as a platform for the culture of human mesenchymal stem cells (hMSCs). The cells were stained for the cell nucleus and actin filaments, as well as immuno-stained for the focal adhesion protein vinculin, and then observed by fluorescence microscopy in order to characterize their spreading behavior. Calcein AM/ethidium homodimer-1 staining allowed the determination of cell viability. The interface between the cells and the NWs was studied using SEM. Results showed that hMSCs underwent a re-organization of actin filaments that translated into a change from an elongated to a spherical cell shape. Actin filaments and vinculin accumulated in bundles, suggesting the attachment and formation of focal adhesion points of the cells on the NWs. Though the overall number of cells attached on the NWs was lower compared to the control, the attached cells maintained a high viability (>90%) for up to 6 d. Analysis of the interface between the NWs and the cells confirmed the re-organization of F-actin and revealed the adhesion points of the cells on the NWs. Additionally, a net of filopodia surrounded each cell, suggesting the probing of the array to find additional adhesion points. The cells maintained their round shape for up to 6 d of culture. Overall, the NW array is a promising nanostructured platform for studying and influencing hMSCs differentiation.


The Mesenchymal Precursor Cell Marker Antibody STRO-1 Binds to Cell Surface Heat Shock Cognate 70.

Fitter S1,2, Gronthos S3,2, Ooi SS1,2, Zannettino AC1,2.

Author information

Abstract

Since its discovery more than 25 years ago, the STRO-1 antibody has played a fundamental role in defining the hierarchical nature of mesenchymal precursor cells (MPC) and their progeny. STRO-1 antibody binding remains a hallmark of immature pluripotent MPC. Despite the significance of STRO-1 in the MPC field, the identity of the antigen has remained elusive. Using a combination of 2-dimensional gel electrophoresis, coupled with Western blotting and Tandem mass spectroscopy, we have identified the STRO-1 antigen as heat shock cognate 70 (HSC70;HSPA8). STRO-1 binds to immune-precipitated HSC70 and siRNA-mediated knockdown of HSPA8 reduced STRO-1 binding. STRO-1 surface binding does not correlate with HSC70 expression and sequestration of cholesterol reduces STRO-1 surface binding, suggesting that the plasma membrane lipid composition may be an important determinant in the presentation of HSC70 on the cell surface. HSC70 is present on the surface of STRO-1+ but not STRO-1− cell lines as assessed by cell surface biotinylation and recombinant HSC70 blocks STRO-1 binding to the cell surface. The STRO-1 epitope on HSC70 was mapped to the ATPase domain using a series of deletion mutants in combination with peptide arrays. Deletion of the first four amino acids of the consensus epitope negated STRO-1 binding. Notably, in addition to HSC70, STRO-1 cross-reacts
with HSP70, however all the clonogenic cell activity is restricted to the STRO-1\textsuperscript{BRIGHT}/HSP70\textsuperscript{-} fraction. These results provide important insight into the properties that define multipotent MPC and provide the impetus to explore the role of cell surface HSC70 in MPC biology.

\textbf{Low-magnitude, high-frequency vibration promotes the adhesion and the osteogenic differentiation of bone marrow-derived mesenchymal stem cells cultured on a hydroxyapatite-coated surface: The direct role of Wnt/β-catenin signaling pathway activation.}

\textbf{Chen B}\textsuperscript{1}, \textbf{Lin T}\textsuperscript{1}, \textbf{Yang X}\textsuperscript{2}, \textbf{Li Y}\textsuperscript{3}, \textbf{Xie D}\textsuperscript{4}, \textbf{Zheng W}\textsuperscript{5}, \textbf{Cui H}\textsuperscript{1}, \textbf{Deng W}\textsuperscript{6}, \textbf{Tan X}\textsuperscript{6}.

\textbf{Author information}

\textbf{Abstract}

The positive effect of low-magnitude, high-frequency (LMHF) vibration on implant osseointegration has been demonstrated; however, the underlying cellular and molecular mechanisms remain unknown. The aim of this study was to explore the effect of LMHF vibration on the adhesion and the osteogenic differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) cultured on hydroxyapatite (HA)-coated surfaces in an in vitro model as well as to elucidate the molecular mechanism responsible for the effects of LMHF vibration on osteogenesis. LMHF vibration resulted in the increased expression of fibronectin, which was measured by immunostaining and RT-qPCR. Stimulation of BMSCs by LMHF vibration resulted in the rearrangement of the actin cytoskeleton with more prominent F-actin. Moreover, the expression of β1 integrin, vinculin and paxillin was notably increased following LMHF stimulation. Scanning electron microscope observations revealed that there were higher cell numbers and more extracellular matrix attached to the HA-coated surface in the LMHF group. Alkaline phosphatase activity as well as the expression of osteogenic-specific genes, namely Runx2, osterix, collagen I and osteocalcin, were significantly elevated in the LMHF group. In addition, the protein expression of Wnt10B, β-catenin, Runx2 and osterix was increased following exposure to LMHF vibration. Taken together, the findings of this study indicate that LMHF vibration promotes the adhesion and the osteogenic differentiation of BMSCs on HA-coated surfaces in vitro, and LMHF vibration may directly induce osteogenesis by activating the Wnt/β-catenin signaling pathway. These data suggest that LMHF vibration enhances the osseointegration of bone to a HA-coated implant, and provide a scientific foundation for improving bone-implant osseointegration through the application of LMHF vibration.

\textbf{ACS Appl Mater Interfaces.} 2016 Dec 27. doi: 10.1021/acsami.6b12437. [Epub ahead of print]

\textbf{Influence of Crosslinkers on the In Vitro Chondrogenesis of Mesenchymal Stem Cells in Hyaluronic Acid Hydrogels.}

\textbf{Maturavongsadit P, Bi X, Metavarayuth K, Luckanagul JA, Wang Q.}

\textbf{Abstract}

This study aims to investigate the effect of the structures of crosslinkers on the in vitro chondrogenic differentiation of bone mesenchymal stem cells (BMSCs) in hyaluronic acid (HA)-based hydrogels. The
hydrogels were prepared by the covalent crosslinking of methacrylated HA with different types of thiol-tailored molecules, including dithiothreitol (DTT), 4-arm polyethylene glycol (PEG), and multi-arm polyamidoamine (PAMAM) dendrimer using thiol-ene “click” chemistry. The microstructure, mechanical properties, diffusivity and degradation rates of the resultant hydrogels were controlled by the structural feature of different crosslinkers. BMSCs were then encapsulated in the resulting hydrogels and cultured in chondrogenic condition. Overall, chondrogenic differentiation was highly enhanced in the PEG-crosslinked HA hydrogels, as measured by glycosaminoglycan (GAG) and collagen accumulation. The physical properties of hydrogels, especially the mechanical property and microarchitecture, were resulted from the structures of different crosslinkers, which subsequently modulated the fate of BMSC differentiation.


Comparative characterization of mesenchymal stromal cells from multiple abdominal adipose tissues and enrichment of angiogenic ability via CD146 molecule.

Lee NE1, Kim SJ2, Yang SJ3, Joo SY4, Park H5, Lee KW5, Yang HM6, Park JB7.

Author information

Abstract

BACKGROUND:
There are various types of adipose tissue in the human body, and their morphology is known to be closely related to cell function and metabolism. However, the functional differences among the mesenchymal stromal cells (MSCs) of different abdominal adipose tissues have not been clearly elucidated.

METHODS:
MSCs were isolated from different abdominal adipose tissues according to their regional distribution and included superficial subcutaneous, deep subcutaneous, omentum, mesentery and retroperitoneal MSCs. The immunophenotype, proliferative ability and angiogenic function of these MSCs were compared based on flow cytometry analysis, CCK-8 proliferation, in vitro differentiation, tubule formation and in vivo plug assay.

RESULTS:
The plastic adherence, cell morphology and general immunophenotype are similar among the MSCs. However, subcutaneous adipose tissue-derived MSCs have a faster growth rate and a higher level of CD146 expression than the other MSCs. Moreover, according to the fluorescence-activated cell sorting (FACS) enrichment procedure, the expression level of CD146 is positively related to the growth rate and angiogenic capability of MSCs.
DISCUSSION:
MSCs in adipose tissue showed slightly different characteristics depending on their location of origin, and they possessed different angiogenic abilities that were mediated by the expression of CD146. This study provides evidence that subcutaneous adipose tissue is the most appropriate source of MSCs for therapeutic cell transplantation in vascular disease.


A clinically relevant hydrogel based on hyaluronic acid and platelet rich plasma as a carrier for mesenchymal stem cells: Rheological and biological characterization.


Author information

Abstract
Intervertebral disc regeneration is quickly moving towards clinical applications. However, it is still missing an ideal injectable hydrogel to support mesenchymal stem cells (MSC) delivery. Herein, a new injectable hydrogel composed of platelet rich plasma (PRP) and hyaluronic acid (HA) blended with batroxobin (BTX) as gelling agent, was designed to generate a clinically relevant cell carrier for disc regeneration. PRP/HA/BTX blend was tested for rheological properties. Amplitude sweep, frequency sweep and rotational measurements were performed and viscoelastic properties were evaluated. Human MSC encapsulated in PRP/HA/BTX hydrogel were cultured in both growing medium and medium with or without TGF-β1 up to day 21. The amount of glycosaminoglycan was evaluated. Quantitative gene expression evaluation for collagen type II, aggrecan and Sox 9 was also performed. Rheological tests showed that the hydrogel jellifies in 15 minutes 20°C and in 3 minutes at 37°C. Biological test showed that MSCs cultured in the hydrogel maintain high cell viability and proliferation. Human MSC within the hydrogel cultured with or without TGF-β1 showed significantly higher GAG production compared to control medium. Moreover, MSCs in the hydrogel underwent differentiation to chondrocyte-like cells with TGF-β1, as shown by histology and gene expression analysis. This novel hydrogel improves viability and proliferation of MSCs supporting the differentiation process toward chondrocyte-like cells. Rheology tests showed optimal gelation kinetics at room temperature for manipulation and faster gelation after transplantation (37°C). The clinical availability of all components of the hydrogel will allow a rapid translation of this regenerative approach into the clinical scenario.


Mitochondrial Functional Changes Characterization in Young and Senescent Human Adipose DerivedMSCs.


Author information

Abstract
Mitochondria are highly dynamic organelles that in response to the cell's bio-energetic state continuously undergo structural remodeling fission and fusion processes. This mitochondrial dynamic activity has been implicated in cell cycle, autophagy, and age-related diseases. Adult tissue-derived mesenchymal stromal/stem cells present a therapeutic potential. However, to obtain an adequate mesenchymal stromal/stem cell number for clinical use, extensive in vitro expansion is required. Unfortunately, these cells undergo replicative senescence rapidly by mechanisms that are not well understood. Senescence has been associated with metabolic changes in the oxidative state of the cell, a process that has been also linked to mitochondrial fission and fusion events, suggesting an association between mitochondrial dynamics and senescence. In the present work, we studied the mitochondrial structural remodeling process of mesenchymal stromal/stem cells isolated from adipose tissue in vitro to determine if mitochondrial phenotypic changes were associated with mesenchymal stromal/stem cell senescence. For this purpose, mitochondrial dynamics and oxidative state of stromal/stem cell were compared between young and old cells. With increased cell passage, we observed a significant change in cell morphology that was associated with an increase in β-galactosidase activity. In addition, old cells (population doubling seven) also showed increased mitochondrial mass, augmented superoxide production, and decreased mitochondrial membrane potential. These changes in morphology were related to slightly levels increases in mitochondrial fusion proteins, Mitofusion 1 (MFN1), and Dynamin-related GTPase (OPA1). Collectively, our results showed that adipose tissue-derived MSCs at population doubling seven developed a senescent phenotype that was characterized by metabolic cell changes that can lead to mitochondrial fusion.


Mitochondrial Functional Changes Characterization in Young and Senescent Human Adipose Derived MSCs.

Stab BR 2nd¹, Martinez L¹, Grismaldo A¹, Lerma A¹, Gutiérrez ML², Barrera LA³, Sutachan JJ¹, Albarracín SL¹.

Author information

Abstract

Mitochondria are highly dynamic organelles that in response to the cell's bio-energetic state continuously undergo structural remodeling fission and fusion processes. This mitochondrial dynamic activity has been implicated in cell cycle, autophagy, and age-related diseases. Adult tissue-derived mesenchymal stromal/stem cells present a therapeutic potential. However, to obtain an adequate mesenchymal stromal/stem cell number for clinical use, extensive in vitro expansion is required. Unfortunately, these cells undergo replicative senescence rapidly by mechanisms that are not well understood. Senescence has been associated with metabolic changes in the oxidative state of the cell, a process that has been also linked to mitochondrial fission and fusion events, suggesting an association between mitochondrial dynamics and senescence. In the present work, we studied the mitochondrial structural remodeling process of mesenchymal stromal/stem cells isolated from adipose tissue in vitro to determine if mitochondrial phenotypic changes were associated with mesenchymal stromal/stem cell senescence. For this purpose, mitochondrial dynamics and oxidative state of
stromal/stem cell were compared between young and old cells. With increased cell passage, we observed a significant change in cell morphology that was associated with an increase in β-galactosidase activity. In addition, old cells (population doubling seven) also showed increased mitochondrial mass, augmented superoxide production, and decreased mitochondrial membrane potential. These changes in morphology were related to slightly levels increases in mitochondrial fusion proteins, Mitofusion 1 (MFN1), and Dynamin-related GTPase (OPA1). Collectively, our results showed that adipose tissue-derived MSCs at population doubling seven developed a senescent phenotype that was characterized by metabolic cell changes that can lead to mitochondrial fusion.


**DMSO-free cryopreservation of adipose-derived mesenchymal stromal cells: expansion medium affects post-thaw survival.**

Rogulska O¹, Petrenko Y²,³, Petrenko A²

**Author information**

**Abstract**

Off-the-shelf availability of human adipose-derived mesenchymal stromal cells (ASCs) for regenerative medicine application requires the development of nontoxic, safe, and efficient protocols for cryopreservation. Favorably, such cell processing protocols should not contain xenogeneic or toxic components, such as fetal bovine serum (FS) and dimethyl sulfoxide (DMSO). The objective of the study was to assess the sensitivity of ASCs to DMSO-free cryopreservation protocol depending on their expansion conditions: conventional, based on the application of FS or xeno-free, using PL as a medium supplement. ASCs expansion was carried out in α-MEM supplemented either with FS or PL. For DMSO- and xeno-free cryopreservation ASCs were pretreated with different concentrations of sucrose during 24 h of culture. Pretreated ASCs were cryopreserved in α-MEM containing 100-300 mM of sucrose with the cooling rate of 1 degree/min. ASCs were tested for survival (Trypan Blue test), viability (MTT test), recovery (Alamar Blue test), proliferation and ability to multilineage differentiation. The optimal concentrations of sucrose for ASCs pretreatment and as an additive in cryoprotective solution, which provided highest cell survival, comprised 100 and 200 mM, correspondingly. Survival and recovery rates of platelet lysate (PL)-expanded ASCs after DMSO-free cryopreservation comprised 59 and 51%, and were higher than in FS-cultured cells. After DMSO-free cryopreservation PL-processed ASCs had a shorter population doubling time and higher capacity for osteogenic differentiation than FS-processed cultures. The described DMSO- and xeno-free processing may form the basis for the development of safe and efficient protocols for manufacturing and banking of ASCs, providing their off-the-shelf availability for regenerative medicine applications.


**Intraarticular and intravenous administration of 99MTc-HMPAO-labeled human mesenchymal stem cells (99MTC-AH-MSCS): In vivo imaging and biodistribution.**
INTRODUCTION:
Therapeutic application of intravenous administered (IV) human bone marrow-derived mesenchymal stem cells (ahMSCs) appears to have as main drawback the massive retention of cells in the lung parenchyma, questioning the suitability of this via of administration. Intraarticular administration (IAR) could be considered as an alternative route for therapy in degenerative and traumatic joint lesions. Our work is outlined as a comparative study of biodistribution of $^{99m}$Tc-ahMSCs after IV and IAR administration, via scintigraphic study in an animal model.

METHODS:
Isolated primary culture of adult human mesenchymal stem cells was labeled with $^{99m}$Tc-HMPAO for scintigraphic study of in vivo distribution after intravenous and intra-articular (knee) administration in rabbits.

RESULTS:
IV administration of radiolabeled ahMSCs showed the bulk of radioactivity in the lung parenchyma while IAR images showed activity mainly in the injected cavity and complete absence of uptake in pulmonary bed.

CONCLUSIONS:
Our study shows that IAR administration overcomes the limitations of IV injection, in particular, those related to cells destruction in the lung parenchyma. After IAR administration, cells remain within the joint cavity, as expected given its size and adhesion properties.

ADVANCES IN KNOWLEDGE:
Intra-articular administration of adult human mesenchymal stem cells could be a suitable route for therapeutic effect in joint lesions.

IMPLICATIONS FOR PATIENT CARE:
Local administration of adult human mesenchymal stem cells could improve their therapeutic effects, minimizing side effects in patients.