ML 16-18 (02/05/2018)

Front Cell Dev Biol. 2018 Apr 4;6:33. doi: 10.3389/fcell.2018.00033. eCollection 2018.

Human Adipose-Derived Mesenchymal Stem/Stromal Cells Handling Protocols. Lipid Droplets and Proteins Double-Staining.

Gojanovich AD[1], Gimenez MC[1,2], Masone D[1,3], Rodriguez TM[4], Dewey RA[4], Delgui LR[1,5], Bustos DM[1,5], Uhart M[1].
Author information

Abstract

Human Adipose-derived mesenchymal stem/stromal cells (hASCs) are of great interest because of their potential for therapeutic approaches. The method described here covers every single step necessary for hASCs isolation from subcutaneous abdominal adipose tissue, multicolor phenotyping by flow cytometry, and quantitative determination of adipogenic differentiation status by means of lipid droplets (LDs) accumulation, and Western blot analysis. Moreover, to simultaneously analyze both LDs accumulation and cellular proteins localization by fluorescence microscopy, we combined Oil Red O (ORO) staining with immunofluorescence detection. For LDs quantification we wrote a program for automatic ORO-stained digital image processing implemented in Octave, a freely available software package. Our method is based on the use of the traditional low cost neutral lipids dye ORO, which can be imaged both by bright-field and fluorescence microscopy. The utilization of ORO instead of other more expensive lipid-specific dyes, together with the fact that the whole method has been designed employing cost-effective culture reagents (standard culture medium and serum), makes it affordable for tight-budget research laboratories. These may be replaced, if necessary or desired, by defined xeno-free reagents for clinical research and applications.

Int Orthop. 2018 Apr 26. doi: 10.1007/s00264-018-3953-4. [Epub ahead of print]

Stem cell therapy in bilateral osteonecrosis: computer-assisted surgery versus conventional fluoroscopic technique on the contralateral side.

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Author information

Abstract

PURPOSE:

Surgical management of osteonecrosis with core decompression with stem cell therapy is a new procedure. The technique is performed with fluoroscopic guidance. This study attempts to determine if computer-navigated technique can improve the procedure.

METHODS:

Thirty consecutive patients with bilateral symptomatic osteonecrosis without collapse were included in this study during the year 2011. A prospective, randomized, and controlled study was conducted on 60

hips (bilateral osteonecrosis) using conventional fluoroscopy technique on one side and computer-based navigation on the contralateral side. Bone marrow aspirated from the two iliac crests was mixed before concentration. Each side received the same volume of concentrated bone marrow and the same number of cells 110,000 ± 27,000 cells (counted as CFU-F).

RESULTS:

Computer navigation achieved better parallelism to the ideal position of the trocar, with better trocar placement as regards to tip-to-subchondral distance and ideal centre position within the osteonecrosis for injection of stem cells. Using computer navigation took fewer attempts to position the trocar, used less fluoroscopy time, and decreased the radiation exposure as compared with surgery performed with conventional fluoroscopy. At the most recent follow-up (6 years), increasing the precision with computer navigation resulted in less collapse (7 versus 1) and better volume of repair (13.4 versus 8.2 cm^3) for hips treated with the computer-assisted technique.

CONCLUSIONS:

The findings of this study suggest that computer navigation may be safely used in a basic procedure for injection of stem cells.

Bone. 2018 Apr 22. pii: S8756-3282(18)30168-6. doi: 10.1016/j.bone.2018.04.014. [Epub ahead of print]

GH prevents adipogenic differentiation of mesenchymal stromal stem cells derived from human trabecular bone via canonical Wnt signaling.

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Author information

Abstract

The imbalance between osteogenesis and adipogenesis, which naturally accompanies bone marrow senescence, may contribute to the development of bone-associated diseases, like osteoporosis. In the present study, using primary human mesenchymal stromal cells (hMSCs) isolated from trabecular bone, we assessed the possible effect of GH on hMSC differentiation potential into adipocytes. GH (5 ng/ml) significantly inhibited the lipid accumulation in hMSCs cultured for 14 days in lipogenic medium. GH decreased the expression of the adipogenic genes, CCAAT/enhancer-binding protein alpha (C/EBPα) and adiponectin (ADN) as well as the expression of two lipogenesis-related enzymes, lipoprotein lipase (LPL) and acethylCoA carboxylase (ACACA). In parallel, GH induced an increase in the gene expression and protein levels of osterix (OSX) and osteoprotegerin (OPG). These effects were ascribed to enhanced Wnt signaling as GH significantly reduced Wnt inhibitors, Dickkopf 1 (DKK1) and the secreted frizzled protein 2 (SFRP2), and increased the expression of an activator of Wnt, Wnt3. Accordingly, the expression of β-catenin and its nuclear levels were raised. Wnt involvement in GH anti-adipogenic effect was further confirmed by the silencing of β-catenin. In silenced hMSC, both the inhibitory effect of GH on the expression of the adipogenic genes, ADN and C/EBPα and the lipogenesis enzymes LPL and ACACA, were prevented together with the stimulatory effect of GH on

the osteogenic genes OSX and OPG. The present study supports the hypothesis that when GH secretion declines as in aging, the fat in the bone-marrow cavities increases and the osteogenic capacity of the MSC pool is reduced due to a decrease in Wnt signaling.

Int J Mol Med. 2018 Apr 19. doi: 10.3892/ijmm.2018.3635. [Epub ahead of print]

Mesenchymal stem cells attenuate doxorubicin-induced cellular senescence through the VEGF/Notch/TGF-β signaling pathway in H9c2 cardiomyocytes.

Chen L[1], Xia W[2], Hou M[3].
Author information

Abstract

The clinical use of doxorubicin (Dox) is limited by its cardiotoxicity. The fundamental changes it induces include interstitial myocardial fibrosis and the appearance of senescent cardiomyocytes. Mesenchymal stem cell (MSC)-based therapies have also been reported to modulate cellular senescence, and have been used effectively to treat age-related cardiovascular diseases. In the present study, the Transwell system was used to coculture H9c2 cells with MSCs, and their proliferation and viability were assessed. The expression of senescence-related genes p53 and p16, and telomere length were measured using reverse transcription-quantitative polymerase chain reaction analysis, and the Jagged-1/Notch-1 signaling pathway was detected using western blot analysis. The results revealed that Dox induced the senescence of H9c2 cells, characterized by a low proliferation rate, poor viability, reduced telomere length and impaired telomerase activity, and by marked increases in the expression of p53 and p16. By contrast, when cocultured with MSCs in the presence of Dox, H9c2 cell proliferation and viability increased, whereas the expression levels of p53 and p16 decreased, and telomere length and telomerase activity increased. The mechanism underlying the antisenescence function of MSCs was clarified, which involved the vascular endothelial growth factor (VEGF)/Jagged-1/Notch-1/transforming growth factor-β1 (TGF-β1) signaling pathway. It was confirmed that inhibiting VEGF, or silencing Jagged-1 or Notch-1 with small interfering RNA, or using recombinant TGF-β1 eliminated the antisenescence effects of MSCs on the Dox-treated H9c2 cells. The results revealed that MSCs rescued H9c2 cells from Dox-induced senescence through the release of VEGF, which activated the Jagged-1/Notch-1 signaling pathway, leading to the inhibition of TGF-β1 release. Therefore, treatment with MSCs may have important therapeutic implications on the attenuation of cardiotoxicity in patients with cancer treated with Dox.

Endocr Metab Immune Disord Drug Targets. 2018 Apr 22. doi: 10.2174/1871530318666180423102905. [Epub ahead of print]

Recent advances in endocrine, metabolic and immune disorders: mesenchymal stem cells (MSCs) and engineered scaffolds.

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Author information

Abstract

BACKGROUND:

New sources of stem cells in adult organisms are constantly emerging. Postnatal Mesenchymal Stem Cells (MSCs), are the most promising support to perform an effective regenerative medicine: such cells have the ability to differentiate into several lineages, such as osteoblasts and chondroblasts, providing novel strategies to improve different complex treatments, during bone regeneration. 3D-printed biomaterials can be designed with geometry aimed to induce stem cells to differentiate towards specific lineage.

OBJECTIVE:

The interaction between stem cells easy to isolate and engineered 3D-printed scaffolds can translate the tissue bio-engineering into bone regenerative surgery. For those reasons, to better identify the complexity represented by the activities and responses of MSCs requires the advance of new target therapies which are not current in endocrine, metabolic and immune disorders and yet to be developed.

METHOD:

This topical review briefly focuses on the new approaches of translational medicine with the use of MSCs and scaffolds engineered with the aid of 3D-printing technology, highlights the osteogenic functions then addressing their applications across the breadth of regenerative medicine.

RESULTS:

The application of bone constructs consisting of engineered scaffold and MSCs as well as the aspects related to the optimal scaffold geometry that favours the best MSCs differentiation and the improvement of concepts as "sensing surface" were also discussed.

CONCLUSION:

Regenerative surgery is largely growing in the field of translational medicine. The use of new sources of MSCs and the improvement of new concepts of bio-engineered scaffolds will certainly be the next step of customized medicine.

Cell Transplant. 2018 Jan 1:963689717723636. doi: 10.1177/0963689717723636. [Epub ahead of print]

Exosomes and Stem Cells in Degenerative Disease Diagnosis and Therapy.

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Author information

Abstract

Stroke can cause death and disability, resulting in a huge burden on society. Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by motor dysfunction. Osteoarthritis (OA) is a progressive degenerative joint disease characterized by cartilage destruction and osteophyte formation in the joints. Stem cell therapy may provide a biological treatment alternative to traditional pharmacological therapy. Mesenchymal stem cells (MSCs) are preferred because of their differentiation ability and possible derivation from many adult tissues. In addition, the paracrine effects of MSCs play

crucial anti-inflammatory and immunosuppressive roles in immune cells. Extracellular vesicles (EVs) are vital mediators of cell-to-cell communication. Exosomes contain various molecules such as microRNA (miRNA), which mediates biological functions through gene regulation. Therefore, exosomes carrying miRNA or other molecules can enhance the therapeutic effects of MSC transplantation. MSC-derived exosomes have been investigated in various animal models representing stroke, PD, and OA. Exosomes are a subtype of EVs. This review article focuses on the mechanism and therapeutic potential of MSC-derived exosomes in stroke, PD, and OA in basic and clinical aspects.

Adv Exp Med Biol. 2018;1058:359-372. doi: 10.1007/978-3-319-76711-6_16.

Stem Cells in Osteochondral Tissue Engineering.

Pintus E[1], Baldassarri M[1], Perazzo L[1], Natali S[1], Ghinelli D[1], Buda R[2].
Author information

Abstract

Mesenchymal stem cells (MSCs) are pluripotent stem cells with the ability to differentiate into a variety of other connective tissue cells, such as chondral, bony, muscular, and tendon tissue. Bone marrow-derived MSCs are pluripotent cells that can differentiate among others into osteoblasts, adipocytes and chondrocytes.Bone marrow-derived cells may represent the future in osteochondral repair. A one-step arthroscopic technique is developed for cartilage repair, using a device to concentrate bone marrow-derived cells and collagen powder or hyaluronic acid membrane as scaffolds for cell support and platelet gel.The rationale of the "one-step technique" is to transplant the entire bone-marrow cellular pool instead of isolated and expanded mesenchymal stem cells allowing cells to be processed directly in the operating room, without the need for a laboratory phase. For an entirely arthroscopic implantation are employed a scaffold and the instrumentation previously applied for ACI; in addition to these devices, autologous platelet-rich fibrin (PRF) is added in order to provide a supplement of growth factors. Results of this technique are encouraging at mid-term although long-term follow-up is still needed.

J Biomed Mater Res A. 2018 Apr 24. doi: 10.1002/jbm.a.36433. [Epub ahead of print]

Non-union Fractures, Mesenchymal Stem Cells and Bone Tissue Engineering.

Toosi S[1,2], Behravan N[3], Behravan J[1,2].
Author information

Abstract

Depending on the duration of healing process, 5-10% of bone fractures may result in either non-union or delayed union. Because non-unions remain a clinically important problem, there is interest in the utilization of tissue engineering strategies to augment bone fracture repair. Three basic biologic elements that are required for bone regeneration include cells, extracellular matrix scaffolds and biological adjuvants for growth, differentiation and angiogenesis. Mesenchymal Stem Cells (MSCs) are capable to differentiate into various types of the cells including chondrocytes, myoblasts, osteoblasts, and adipocytes. Due to their potential for multilineage differentiation, MSCs are considered important

contributors in bone tissue engineering research. In this review we highlight the progress in the application of biomaterials, stem cells and tissue engineering in promoting non-union bone fracture healing.

Methods Mol Biol. 2018;1773:155-165. doi: 10.1007/978-1-4939-7799-4_13.

Isolation of Human Adipose-Derived Stem Cells from Lipoaspirates.

Li J[1,2], Curley JL[1,2], Floyd ZE[3], Wu X[1,2], Halvorsen YDC[4], Gimble JM[5,6].
Author information

Abstract

Adipose tissue is as an abundant and accessible source of stem cells with multipotent properties suitable for tissue engineering and regenerative medical applications. Here, we describe methods from our own laboratory and the literature for the isolation and expansion of adipose-derived stem cells (ASCs). We present a large scale procedure suitable for processing >100 mL volumes of lipoaspirate tissue specimens by collagenase digestion and a related procedure suitable for processing adipose tissue aspirates without digestion.

Methods Mol Biol. 2018;1773:137-146. doi: 10.1007/978-1-4939-7799-4_11.

Isolation of Murine Adipose-Derived Stromal/Stem Cells for Adipogenic Differentiation or Flow Cytometry-Based Analysis.

Kilroy G[1], Dietrich M[1], Wu X[2], Gimble JM[2], Floyd ZE[3].
Author information

Abstract

Murine models of obesity or reduced adiposity are a valuable resource for understanding the role of adipocyte dysfunction in metabolic disorders. Adipose tissue stromal vascular cells or primary adipocytes derived from murine adipose tissue and grown in culture are essential tools for studying the mechanisms underlying adipocyte development and function. Herein, we describe methods for the isolation, expansion, and long-term storage of murine adipose-derived stromal/stem cells along with protocols for inducing adipogenesis in this cell population or isolating the adipose stromal vascular fraction cells for flow cytometric analysis.

Methods Mol Biol. 2018;1773:107-122. doi: 10.1007/978-1-4939-7799-4_9.

Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Implications for Tissue Engineering Therapies in Regenerative Surgery.

Gentile P[1,2], Cervelli V[3].
Author information

Abstract

Cell-based therapy and regenerative medicine offer a paradigm shift in regard to various diseases causing loss of substance or volume and tissue or organ damage. Recently, many authors have

focused their attention on mesenchymal stem cells for their capacity to differentiate into many cell lineages. The most widely studied types are bone marrow mesenchymal stem cells and adipose derived stem cells (ADSCs), which display similar results. Based on the literature, we believe that the ADSCs offer advantages because of lower morbidity during the harvesting procedure. Additionally, platelet-rich plasma can be used in this field for its ability to stimulate tissue regeneration. The aim of this chapter is to describe ADSC preparation and isolation procedures, preparation of platelet-rich plasma, and the application of ADSCs in regenerative plastic surgery. We also discuss the mechanisms and future role of ADSCs in cell-based therapy and tissue engineering.

Cell Transplant. 2018 Jan 1:963689717745890. doi: 10.1177/0963689717745890. [Epub ahead of print]

Aging- and Senescence-associated Changes of Mesenchymal Stromal Cells in Myelodysplastic Syndromes.

Mattiucci D[1], Maurizi G[1], Leoni P[1], Poloni A[1].
Author information

Abstract

Hematopoietic stem and progenitor cells reside within the bone marrow (BM) microenvironment. By a well-balanced interplay between self-renewal and differentiation, they ensure a lifelong supply of mature blood cells. Physiologically, multiple different cell types contribute to the regulation of stem and progenitor cells in the BM microenvironment by cell-extrinsic and cell-intrinsic mechanisms. During the last decades, mesenchymal stromal cells (MSCs) have been identified as one of the main cellular components of the BM microenvironment holding an indispensable role for normal hematopoiesis. During aging, MSCs diminish their functional and regenerative capacities and in some cases encounter replicative senescence, promoting inflammation and cancer progression. It is now evident that alterations in specific stromal cells that comprise the BM microenvironment can contribute to hematologic malignancies, and there is growing interest regarding the contribution of MSCs to the pathogenesis of myelodysplastic syndromes (MDSs), a clonal hematological disorder, occurring mostly in the elderly, characterized by ineffective hematopoiesis and increased tendency to acute myeloid leukemia evolution. The pathogenesis of MDS has been associated with specific genetic and epigenetic events occurring both in hematopoietic stem cells (HSCs) and in the whole BM microenvironment with an aberrant cross talk between hematopoietic elements and stromal compartment. This review highlights the role of MSCs in MDS showing functional and molecular alterations such as altered cell-cycle regulation with impaired proliferative potential, dysregulated cytokine secretion, and an abnormal gene expression profile. Here, the current knowledge of impaired functional properties of both aged MSCs and MSCs in MDS have been described with a special focus on inflammation and senescence induced changes in the BM microenvironment. Furthermore, a better understanding of aberrant BM microenvironment could improve future potential therapies.

Methods Mol Biol. 2018;1758:139-149. doi: 10.1007/978-1-4939-7741-3_11.

High-Throughput Formation of Mesenchymal Stem Cell Spheroids and Entrapment in Alginate Hydrogels.

Vorwald CE[1], Ho SS[1], Whitehead J[1], Leach JK[2,3].
Author information

Abstract

Mesenchymal stem cells (MSCs) are a promising cell source for tissue repair and regeneration due to their multilineage capacity, potential for autologous use, and secretion of potent bioactive factors to catalyze the endogenous repair program. However, a major limitation to current cell-based tissue engineering approaches is the drastic loss of cells upon transplantation. The causation of this loss, whether due to apoptosis following a dramatic change in the microenvironment or migration away from the defect site, has yet to be determined. MSCs formed into aggregates, known as spheroids, possess a strong therapeutic advantage compared to the more commonly used dissociated cells due to their improved resistance to apoptosis and increased secretion of endogenous trophic factors. Furthermore, the use of biomaterials such as alginate hydrogels to transplant cells in situ improves cell survival, localizes payloads at the defect site, and facilitates continued instruction of cells by manipulating the biophysical properties of the biomaterial. Transplantation of MSC spheroids without a vehicle into tissue defects comprises the majority of studies to date, ceding control of spheroid function due to the cell's interaction with the native tissue extracellular matrix and abrogating the established benefits of spheroid formation. Thus, there is a significant need to consider the role of biomaterials in transplanting MSC spheroids using an appropriate carrier. In this chapter, we describe high-throughput formation of spheroids, steps for further characterization, and encapsulation in alginate hydrogels with an eye toward localizing MSC spheroids at the target site.

Clin Exp Immunol. 2018 Apr 20. doi: 10.1111/cei.13141. [Epub ahead of print]

Indirect co-cultures of healthy mesenchymal stem cells restore the physiological phenotypic profile of psoriatic mesenchymal stem cells.

Campanati A[1], Orciani M[2], Sorgentoni G[2], Consales V[1], Mattioli Belmonte M[2], Di Primio R[2], Offidani A[1].
Author information

Abstract

Psoriasis microenvironment, characterized by an imbalance between Th1/Th17 and Th2 cytokines, influences also mesenchymal stem cells (MSCs) phenotypic profile. MSCs from healthy donors (H-MSCs) can exert a strong paracrine effect by secreting active soluble factors, able to modulate the inflammation in the microenvironment. To evaluate the influence of H-MSCs on MSCs from psoriatic patients (PsO-MSCs), H-MSCs and PsO-MSCs were isolated and characterized. Indirect co-culture of H-MSCs with PsO-MSCs was performed; effects on proliferation and expression of cytokines linked to Th1/Th17 and Th2 pathways were assayed before and after co-culture. The results show that before co-culture, proliferation of PsO-MSCs was significantly higher than H-MSCs ($p<0.05$) and the levels of secreted cytokines confirmed the imbalance of Th1/17 versus Th2 axis. After co-culture of H-MSCs with

PsO-MSCs, healthy MSCs seem to exert a "positive" influence on PsO-MSCs driving the inflammatory phenotypic profile of PsO-MSCs towards a physiological pattern. The proliferation rate decreased, towards values nearer to those observed in H-MSCs and the secretion of the cytokines that mostly identified the inflammatory microenvironment that characterized psoriasis, such as IL6, IL12, IL13, IL17A, TNFα, and GCSF, is significantly lower in co-cultured PsO-MSCs than in individually cultured PSO-MSCs (p at least <0.05). In conclusions, our preliminary results seem to provide an intriguing molecular explanation for the ever increasing evidence of therapeutic efficacy of allogeneic MSCs infusion in psoriatic patients

Tissue Eng Part C Methods. 2018 Apr 20. doi: 10.1089/ten.TEC.2017.0518. [Epub ahead of print]

Housekeeping gene stability in human mesenchymal stem and tendon cells exposed to tenogenic factors.

Viganò M[1], Perucca Orfei C[2], de Girolamo L[3], Pearson J[4], Ragni E[5], De Luca P[6], Colombini A[7].
Author information

Abstract

The use of biochemical inducers of mesenchymal stem cells (MSCs) differentiation into tenogenic lineage represents an investigated aspect of tendon disorder treatment. Bone morphogenetic protein 12 (BMP-12) is a widely studied factor, representing along with ascorbic acid (AA) and basic fibroblast growth factor (bFGF) one of the most promising stimulus in this context so far. Quantitative gene expression of specific tenogenic marker is commonly used to assess the efficacy of these supplements. Nevertheless, the reliability of these data is strongly associated with the choice of stable housekeeping genes. To date, no published studies have evaluated the stability of housekeeping genes in MSCs during tenogenic induction. Three candidate housekeeping genes (YWHAZ, RPL13A and GAPDH) in human MSCs from bone marrow (BMSCs), adipose tissue (ASCs) and tendon cells (TCs) supplemented with BMP-12 or AA and bFGF in comparison with control untreated cells for 3 and 10 days were evaluated. GeNorm, NormFinder, BestKeeper tools and the comparative ΔCt method were used to evaluate housekeeping gene stability and the overall ranking was determined by using by the RefFinder algorithm. In all culture conditions, YWHAZ was the most stable gene and RPL13A was the second choice. YWHAZ and RPL13A were the two most stable genes also for ASCs and BMSCs, regardless of the time-point analyzed, and for TCs at 10 days of tenogenic induction. Only for TCs at 3 days of tenogenic induction were GAPDH and YWHAZ the best performers. In conclusion, our findings will be useful for the proper selection of housekeeping genes in studies involving MSCs cultured in presence of tenogenic factors, in order to obtain accurate and high quality data from quantitative gene expression analysis.

Joints. 2018 Feb 12;6(1):16-22. doi: 10.1055/s-0038-1626740. eCollection 2018 Mar.

Survival Analysis after Core Decompression in Association with Platelet-Rich Plasma, Mesenchymal Stem Cells, and

Synthetic Bone Graft in Patients with Osteonecrosis of the Femoral Head.

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Author information

Abstract

Purpose The aim of this study was to report the rate of survivorship in patients with osteonecrosis of the femoral head treated with core decompression in association with mesenchymal stem cells (MSCs) implantation, platelet-rich plasma (PRP) injection, and synthetic bone graft. **Methods** We evaluated 24 hips in 16 patients, according to Ficat classification, treated by core decompression, injection of PRP and MSCs, and backfilling of the core tract with synthetic bone graft. Survivorship was estimated using Kaplan-Meier curves. **Results** The survivorship of core decompression in association with the procedure is 50% at 75 months of follow-up. The survival rate was 80% for patients in early stage and 28.6% for patients in advanced stage at 75 months. When we compared Kaplan-Meier survival curves of patients in stage III + IV and patients in stage I + II, we noticed that the survival functions are statistically different ($p < 0.05$, log-rank test), particularly in stage I + II where we had a greater surviving core decompression, in comparison to patients in stage III + IV. **Conclusion** This technique is safe and good preliminary results were obtained in patients with early stages of the disease with no reported complications. **Level of Evidence** Level IV, therapeutic case series.

Joints. 2018 Mar 13;6(1):4-9. doi: 10.1055/s-0038-1636948. eCollection 2018 Mar.

Migration of Mesenchymal Stem Cells of Bursal Tissue after Rotator Cuff Repair in Rats.

Safi E[1], Ficklscherer A[1], Bondarava M[1], Betz O[1], Zhang A[1], Jansson V[1], Müller PE[1].
Author information

Abstract

Purpose The purpose of this study is to verify migration of mesenchymal stem cells of bursal tissue into the healing site after rotator cuff repair in rats. **Methods** Fischer rats and green fluorescent protein (GFP)-transgenic rats were used. Bursal tissue from GFP rats was isolated and transplanted into tendon repair sites in Fischer rats. We examined the histology of the rotator cuff and the proportion of GFP-positive cells in the repaired rotator cuff 1, 3, and 6 weeks after surgery. **Results** Cell migration was observed during the third and sixth week after surgery. We also found mesenchymal stem cells and formed bursal cluster patterns in the repaired rotator cuff tendons. **Conclusion** Mesenchymal stem cells migrated from bursal tissue and infiltrated the repaired rotator cuff tendons. **Clinical Relevance** Mesenchymal stem cells from bursal tissue can contribute to the healing progress of the repaired rotator cuff.

NPJ Regen Med. 2018 Apr 17;3:9. doi: 10.1038/s41536-018-0048-1. eCollection 2018.

Translation of remote control regenerative technologies for bone repair.

Markides H[1], McLaren JS[2], Telling ND[1], Alom N[2], Al-Mutheffer EA[1], Oreffo ROC[3], Zannettino A[4], Scammell BE[5], White LJ[6], El Haj AJ[1].
Author information
Abstract

The role of biomechanical stimuli, or mechanotransduction, in normal bone homeostasis and repair is understood to facilitate effective osteogenesis of mesenchymal stem cells (MSCs) in vitro. Mechanotransduction has been integrated into a multitude of in vitro bone tissue engineering strategies and provides an effective means of controlling cell behaviour towards therapeutic outcomes. However, the delivery of mechanical stimuli to exogenous MSC populations, post implantation, poses a significant translational hurdle. Here, we describe an innovative bio-magnetic strategy, MICA, where magnetic nanoparticles (MNPs) are used to remotely deliver mechanical stimuli to the mechano-receptor, TREK-1, resulting in activation and downstream signalling via an external magnetic array. In these studies, we have translated MICA to a pre-clinical ovine model of bone injury to evaluate functional bone repair. We describe the development of a magnetic array capable of in vivo MNP manipulation and subsequent osteogenesis at equivalent field strengths in vitro. We further demonstrate that the viability of MICA-activated MSCs in vivo is unaffected 48 h post implantation. We present evidence to support early accelerated repair and preliminary enhanced bone growth in MICA-activated defects within individuals compared to internal controls. The variability in donor responses to MICA-activation was evaluated in vitro revealing that donors with poor osteogenic potential were most improved by MICA-activation. Our results demonstrate a clear relationship between responders to MICA in vitro and in vivo. These unique experiments offer exciting clinical applications for cell-based therapies as a practical in vivo source of dynamic loading, in real-time, in the absence of pharmacological agents.

J Vet Med Sci. 2018 Apr 18. doi: 10.1292/jvms.17-0563. [Epub ahead of print]

Altered properties of feline adipose-derived mesenchymal stem cells during continuous in vitro cultivation.

Lee BY[1], Li Q[1], Song WJ[1], Chae HK[1], Kweon K[1], Ahn JO[1], Youn HY[1].
Author information
Abstract

Cytotherapy with mesenchymal stem cells (MSCs) has been studied in many species, and often requires in vitro cell expansion to obtain therapeutic doses of stem cells. Because the characteristics of MSCs, such as self-renewal and multi-lineage differentiation, can be altered by long-term culture, it is important to maintain stemness during cultivation. This study assessed the changes in the characteristics of feline adipose tissue-derived (fAT)-MSCs during in vitro passaging. Stem cells isolated from the adipose tissue of donor cats were cultured for seven sub-passages. Proliferation capacity was analyzed by calculating the cell doubling time and by colorimetric assay. Expression of stem cell-specific markers was evaluated by quantitative reverse transcription (qRT)-PCR and immunophenotyping. Expression of adipogenic and osteogenic differentiation markers was also measured by qRT-PCR. Histochemical staining and measurement of β-galactosidase activity were conducted to detect cellular senescence. The cell proliferation rate decreased significantly at passage 5

(P5). Gene expression levels of pluripotency markers (Sox2, Nanog, and Klf4) and stem cell surface markers (CD9, CD44, CD90, and CD105) decreased during continuous culture; in most assays, statistically significant changes were observed at P5. The ability of cells to undergo adipogenic or osteogenic differentiation was inversely proportional to the number of passages. The proportion of senescent cells increased with the number of passages. These results suggest that repeated passages alter the proliferation and multipotency of fAT-MSCs. In clinical trials, early-passage cells should be used to achieve the maximum therapeutic effect.

Proc Natl Acad Sci U S A. 2018 Apr 16. pii: 201720658. doi: 10.1073/pnas.1720658115. [Epub ahead of print]

Developmentally inspired programming of adult human mesenchymal stromal cells toward stable chondrogenesis.

Occhetta P[1], Pigeot S[1], Rasponi M[2], Dasen B[1], Mehrkens A[1], Ullrich T[3], Kramer I[4], Guth-Gundel S[4], Barbero A[1], Martin I[5].
Author information

Abstract

It is generally accepted that adult human bone marrow-derived mesenchymal stromal cells (hMSCs) are default committed toward osteogenesis. Even when induced to chondrogenesis, hMSCs typically form hypertrophic cartilage that undergoes endochondral ossification. Because embryonic mesenchyme is obviously competent to generate phenotypically stable cartilage, it is questioned whether there is a correspondence between mesenchymal progenitor compartments during development and in adulthood. Here we tested whether forcing specific early events of articular cartilage development can program hMSC fate toward stable chondrogenesis. Inspired by recent findings that spatial restriction of bone morphogenetic protein (BMP) signaling guides embryonic progenitors toward articular cartilage formation, we hypothesized that selective inhibition of BMP drives the phenotypic stability of hMSC-derived chondrocytes. Two BMP type I receptor-biased kinase inhibitors were screened in a microfluidic platform for their time- and dose-dependent effect on hMSC chondrogenesis. The different receptor selectivity profile of tested compounds allowed demonstration that transient blockade of both ALK2 and ALK3 receptors, while permissive to hMSC cartilage formation, is necessary and sufficient to maintain a stable chondrocyte phenotype. Remarkably, even upon compound removal, hMSCs were no longer competent to undergo hypertrophy in vitro and endochondral ossification in vivo, indicating the onset of a constitutive change. Our findings demonstrate that adult hMSCs effectively share properties of embryonic mesenchyme in the formation of transient but also of stable cartilage. This opens potential pharmacological strategies to articular cartilage regeneration and more broadly indicates the relevance of developmentally inspired protocols to control the fate of adult progenitor cell systems.

J Orthop Translat. 2017 Mar 29;9:19-27. doi: 10.1016/j.jot.2017.03.002. eCollection 2017 Apr.

Mesenchymal stem cells homing to improve bone healing.

Lin W[1], Xu L[1], Zwingenberger S[2], Gibon E[3,4], Goodman SB[4], Li G[1,5,6].

Author information

Abstract

Cell therapy continues to attract growing interest as a promising approach to treat a variety of diseases. Mesenchymal stem cells (MSCs) have been one of the most intensely studied candidates for cell therapy. Since the homing capacity of MSCs is an important determinant of effective MSC-based therapy, the enhancement of homing efficiency is essential for optimizing the therapeutic outcome. Furthermore, trafficking of endogenous MSCs to damaged tissues, also referred to as endogenic stem cell homing, and the subsequent participation of MSCs in tissue regeneration are considered to be a natural self-healing response. Therefore, strategies to stimulate and reinforce the mobilisation and homing of MSCs have become a key point in regenerative medicine. The current review focuses on advances in the mechanisms and factors governing trafficking of MSCs, and the relationship between MSC mobilisation and skeletal diseases, providing insights into strategies for their potential translational implications.

Scand J Clin Lab Invest. 2018 Apr 17:1-8. doi: 10.1080/00365513.2018.1462082. [Epub ahead of print]

Development of large-scale manufacturing of adipose-derived stromal cells for clinical applications using bioreactors and human platelet lysate.

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Abstract

In vitro expanded adipose-derived stromal cells (ASCs) are a useful resource for tissue regeneration. Translation of small-scale autologous cell production into a large-scale, allogeneic production process for clinical applications necessitates well-chosen raw materials and cell culture platform. We compare the use of clinical-grade human platelet lysate (hPL) and fetal bovine serum (FBS) as growth supplements for ASC expansion in the automated, closed hollow fibre quantum cell expansion system (bioreactor). Stromal vascular fractions were isolated from human subcutaneous abdominal fat. In average, 95 × 10^6 cells were suspended in 10% FBS or 5% hPL medium, and loaded into a bioreactor coated with cryoprecipitate. ASCs (P0) were harvested, and 30 × 10^6 ASCs were reloaded for continued expansion (P1). Feeding rate and time of harvest was guided by metabolic monitoring. Viability, sterility, purity, differentiation capacity, and genomic stability of ASCs P1 were determined. Cultivation of SVF in hPL medium for in average nine days, yielded 546 × 10^6 ASCs compared to 111 × 10^6 ASCs, after 17 days in FBS medium. ASCs P1 yields were in average 605 × 10^6 ASCs (PD [population doublings]: 4.65) after six days in hPL medium, compared to 119 × 10^6 ASCs (PD: 2.45) in FBS medium, after 21 days. ASCs fulfilled ISCT criteria and demonstrated genomic stability and sterility. The use of hPL as a growth supplement for ASCs expansion in the quantum cell expansion system provides an efficient expansion process compared to the use of FBS, while maintaining cell quality appropriate for clinical use. The described process is an obvious choice for manufacturing of large-scale allogeneic ASC products.

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Visualization and Quantification of Mesenchymal Cell Adipogenic Differentiation Potential with a Lineage Specific Marker.

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Abstract

Several dyes are currently available for use in detecting differentiation of mesenchymal cells into adipocytes. Dyes, such as Oil Red O, are cheap, easy to use and widely utilized by laboratories analyzing the adipogenic potential of mesenchymal cells. However, they are not specific to changes in gene transcription. We have developed a gene-specific differentiation assay to analyze when a mesenchymal cell has switched its fate to an adipogenic lineage. Immuno-labelling against fatty acid binding protein-4 (FABP4), a lineage-specific marker of adipogenic differentiation, enabled visualization and quantification of differentiated cells. The ability to quantify adipogenic differentiation potential of mesenchymal cells in a 96 well microplate format has promising implications for a number of applications. Hundreds of clinical trials involve the use of adult mesenchymal stromal cells and it is currently difficult to correlate therapeutic outcomes within and especially between such clinical trials. This simple high-throughput FABP4 assay provides a quantitative assay for assessing the differentiation potential of patient-derived cells and is a robust tool for comparing different isolation and expansion methods. This is particularly important given the increasing recognition of the heterogeneity of the cells being administered to patients in mesenchymal cell products. The assay also has potential utility in high throughput drug screening, particularly in obesity and pre-diabetes research.