ML 06-19 (18/02/2019)

J Extracell Vesicles. 2019 Jan 27;8(1):1568780. doi: 10.1080/20013078.2019.1568780. eCollection 2019.

Raman spectroscopy as a quick tool to assess purity of extracellular vesicle preparations and predict their functionality.

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Author information

Abstract

Extracellular vesicles (EVs) from a variety of stem cell sources are believed to harbour regenerative capacity, which may be exploited for therapeutic purposes. Because of EV interaction with other soluble secreted factors, EV activity may depend on the employed purification method, which limits cross-study comparisons and therapeutic development. Raman spectroscopy (RS) is a quick and easy method to assess EV purity and composition, giving in-depth biochemical overview on EV preparation. Hereby, we show how this method can be used to characterise EVs isolated from human liver stem cells and bone marrow mesenchymal stem/stromal cells by means of conventional ultracentrifugation (UC) and size exclusion chromatography (SEC) protocols. The obtained EV preparations were demonstrated to be characterised by different degrees of purity and a specific Raman fingerprint that represents both the cell source and the isolation procedure used. Moreover, RS provided useful hints to explore the factors underlying the functional diversity of EV preparations from the same cell source, thus representing a valuable tool to assess EV quality prior to functional assays or therapeutic application.

Cancers (Basel). 2019 Feb 5;11(2). pii: E185. doi: 10.3390/cancers11020185.

In Vivo Cell Fusion between Mesenchymal Stroma/Stem-Like Cells and Breast Cancer Cells.

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Author information

Abstract

Cellular communication within the tumor microenvironment enables important interactions between cancer cells and recruited adjacent populations including mesenchymal stroma/stem-like cells (MSC). These interactions were monitored in vivo following co-injection of GFP-labeled human MSC together with mcherry-labeled MDA-MB-231 breast cancer cells in NODscid mice. Within 14 days of tumor development the number of initially co-injected MSC had significantly declined and spontaneous formation of breast cancer/MSC hybrid cells was observed by the appearance of double fluorescing cells. This in vivo fusion displayed a rare event and occurred in less than 0.5% of the tumor cell population. Similar findings were observed in a parallel in vitro co-culture. Characterization of the new cell fusion products obtained after two consecutive flow cytometry cell sorting and single cell cloning revealed two populations, termed MDA-hyb3 and MDA-hyb4. The breast cancer fusion cells expressed both, GFP and mcherry and displayed more characteristics of the MDA-MB-231 cells than of the

parental MSC. While little if any differences were determined in the proliferative capacity, a significant delay of MDA-hyb3 cells in tumor formation was observed when compared to the parental MDA-MB-231 cells. Moreover, MDA-hyb3 cells developed an altered pattern of distant organ metastases. These findings demonstrated dynamic tumor changes by in vivo and in vitro fusion with the development of new breast cancer hybrid cells carrying altered tumorigenic properties. Consequently, cancer cell fusion contributes to progressively increasing tumor heterogeneity which complicates a therapeutic regimen.

Nanomedicine. 2019 Feb 11. pii: S1549-9634(19)30020-6. doi: 10.1016/j.nano.2019.01.008. [Epub ahead of print]

Effects of titania nanotube surfaces on osteogenic differentiation of human adipose-derived stem cells.

Cowden K[1], Dias-Netipanyj MF[2], Popat KC[3].
Author information

Abstract

The surface of an implant is important for successful osseointegration and long-term stability as it can aid in cell migration and proliferation, cell differentiation and allow extracellular matrix production. Earlier studies have shown that nanostructuring the surface of titanium can enhance mesenchymal stem cell (MSC) migration, proliferation, and differentiation. Although many studies have evaluated MSC response on nanostructured surfaces, there are only a few studies that have explored the response of adipose-derived stem cells (ADSC) on titania nanotube surfaces. Because ADSC exhibit great potential in regenerative medicine and have already proven effective in developing new treatments, this study aims to further understand how ADSC interact with titania nanotube surfaces. The results of this study indicate that titania nanotube surfaces enhance ADSC proliferation and differentiation that is also dependent on the size of nanotubes. Additionally, the favorable response of ADSC on nantoube surfaces suggests a potential application in orthopedic tissue regeneration.

Regen Med. 2019 Feb 14. doi: 10.2217/rme-2018-0161. [Epub ahead of print]

Adipose-derived mesenchymal stem cell therapy in the treatment of knee osteoarthritis: a randomized controlled trial.

Freitag J[1,2,3], Bates D[2,3], Wickham J[1], Shah K[3,4], Huguenin L[2,3], Tenen A[2,3,5], Paterson K[6], Boyd R[3,7].

Author information

Abstract

AIM:

To evaluate the efficacy of autologous adipose-derived mesenchymal stem cell (ADMSC) therapy on pain, function and disease modification in knee osteoarthritis.

METHODS:

30 participants with symptomatic knee osteoarthritis were randomized into three groups. Two treatment groups received intra-articular ADMSC therapy consisting of either a single injection

(100 × 10^6 ADMSCs) or two injections (100 × 10^6 ADMSCs at baseline and 6 months). The third group served as control and continued conservative management.

RESULTS:

No serious adverse events were observed. Both treatment groups receiving ADMSCs showed clinically significant pain and functional improvement at completion of follow-up at 12 months. Radiological analysis using the Magnetic Resonance Imaging Osteoarthritis Knee Score indicated modification of disease progression.

CONCLUSION:

Autologous ADMSC therapy appears to be a safe and effective therapy for knee osteoarthritis and may have the potential to prevent disease progression.

Front Bioeng Biotechnol. 2019 Jan 29;7:9. doi: 10.3389/fbioe.2019.00009. eCollection 2019.

Mesenchymal Stem Cell Therapy for Osteoarthritis: The Critical Role of the Cell Secretome.

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Author information

Abstract

Osteoarthritis (OA) is an inflammatory condition still lacking effective treatments. Mesenchymal stem/stromal cells (MSCs) have been successfully employed in pre-clinical models aiming to resurface the degenerated cartilage. In early-phase clinical trials, intra-articular (IA) administration of MSCs leads to pain reduction and cartilage protection or healing. However, the consistent lack of engraftment indicates that the observed effect is delivered through a "hit-and-run" mechanism, by a temporal release of paracrine molecules. MSCs express a variety of chemokines and cytokines that aid in repair of degraded tissue, restoration of normal tissue metabolism and, most importantly, counteracting inflammation. Secretion of therapeutic factors is increased upon licensing by inflammatory signals or apoptosis, induced by the host immune system. Trophic effectors are released as soluble molecules or carried by extracellular vesicles (ECVs). This review provides an overview of the functions and mechanisms of MSC-secreted molecules found to be upregulated in models of OA, whether using *in vitro* or *in vivo* models.

Arch Orthop Trauma Surg. 2019 Feb 11. doi: 10.1007/s00402-019-03140-8. [Epub ahead of print]

Intra-articular injection of mesenchymal stem cells for clinical outcomes and cartilage repair in osteoarthritis of the knee: a meta-analysis of randomized controlled trials.

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Author information

Abstract

INTRODUCTION:

Mesenchymal stem cells (MSCs) have gained popularity for articular cartilage repair. However, efficacy of intra-articular MSCs in osteoarthritis remains unclear. In the setting of a meta-analysis of randomized controlled trials (RCTs), we aimed to investigate the efficacy of intra-articular MSCs on clinical outcomes and cartilage repair in patients with knee osteoarthritis.

MATERIALS AND METHODS:

PubMed, EMBASE, Cochrane Library, CINAHL, and Scopus were searched from inception to March 31, 2017. This study included RCTs using cell population containing MSCs for treatment of knee osteoarthritis. The quality was assessed by Cochrane Collaboration`s risk of bias tool. For meta-analysis, data on clinical outcomes measured by visual analog scale (VAS), Lysholm score, WOMAC and data on cartilage repair measured by MOCART and WORMS were extracted. In studies with several cell concentrations, outcomes of recommended concentration were used mainly to ensure robustness.

RESULTS:

A total of five RCTs (220 patients) were included. Two studies were deemed to have low risk of bias. In pooled analysis, there was significant difference in VAS score (mean difference [MD], - 9.2; 95% CI: - 17.21, - 1.20) and Lysholm score (MD, 8.70; 95% CI 0.06, 17.34), but not WOMAC (MD, - 7.44; 95% CI - 20.38, 5.50). In cumulative functional analysis using Lysholm score and WOMAC in recommended concentration, there was a significant improvement (standard mean difference [SMD], 0.53; 95% CI 0.13, 0.94) after treatment. In cartilage repair assessed by MRI, there was no significant difference (SMD, 0.53; 95% CI- 0.28, 1.34).

CONCLUSIONS:

This meta-analysis demonstrated that intra-articular MSCs have a limited evidence in pain relief and functional improvement in knee osteoarthritis. While MSCs may result in favorable clinical outcomes with a recommended concentration, use of concomitant treatment should be considered. In addition, current evidence does not support the use of intra-articular MSCs for improving cartilage repair in knee osteoarthritis.

LEVEL OF EVIDENCE:

Systematic review of Level-II studies.

Stem Cells Int. 2019 Jan 14;2019:1516746. doi: 10.1155/2019/1516746. eCollection 2019.

Oral Plaque from Type 2 Diabetic Patients Reduces the Clonogenic Capacity of Dental Pulp-Derived Mesenchymal Stem Cells.

Bordin A[1], Pagano F[1], Scaccia E[1], Saccucci M[2], Vozza I[2], Incerti N[1], Polimeni A[2], Cavarretta E[1], Chimenti I[1], De Falco E[1].

Author information

Abstract

Type 2 diabetes (T2D) is a major metabolic disease and a key epigenetic risk factor for the development of additional clinical complications. Among them, periodontitis (PD), a severe inflammatory disease ascribable to a dysregulated physiology and composition of the oral microbiota, represents one of the most relevant complications. Periodontitis can impact the structure of the tooth and likely the stem and progenitor cell pool, which actively contributes to the periodontal microenvironment and homeostasis. Modifications of the oral plaque play a key role in the etiopathogenesis of PD caused by T2D. However, to what extent the biology of the progenitor pool is affected has still to be elucidated. In this short report, we aimed to explore the biological effects of oral plaque derived from T2D patients with PD in comparison to non-diabetic patients with PD. Oral plaque samples were isolated from T2D and non-diabetic subjects with PD. Dental pulp stem cells (DPSCs), derived from the premolar tooth, were conditioned for 21 days with oral plaque samples and tested for their clonogenic ability. Cultures were also induced to differentiate towards the osteogenic lineage, and ALP and osteocalcin gene expression levels were evaluated by real-time qPCR. Results have shown that the number of clones generated by DPSCs exposed to T2D oral plaque was significantly lower compared to controls (ctl). The multivariate analysis confirmed that the decreased clonogenesis was significantly correlated only with T2D diagnosis. Moreover, the effect of T2D oral plaque was specific to DPSCs. Indicators of osteogenic differentiation were not significantly affected. This study provides a new biological insight into the effects ascribable to T2D in PD.

PLoS One. 2019 Feb 12;14(2):e0212192. doi: 10.1371/journal.pone.0212192. eCollection 2019.

Tenogenic differentiation protocol in xenogenic-free media enhances tendon-related marker expression in ASCs.

Stanco D[1,2], Caprara C[1], Ciardelli G[2], Mariotta L[1], Gola M[1], Minonzio G[1], Soldati G[1].
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Abstract

Adipose-derived stem cells (ASCs) are multipotent and immune-privileged mesenchymal cells, making them ideal candidates for therapeutic purposes to manage tendon disorders. Providing safe and regulated cell therapy products to patients requires adherence to good manufacturing practices. To this aim we investigated the in vitro tenogenic differentiation potential of ASCs using a chemically defined serum-free medium (SF) or a xenogenic-free human pooled platelet lysate medium (hPL) suitable for cell therapy and both supplemented with CTGF, TGFβ-3, BMP-12 and ascorbic acid (AA) soluble factors. Human ASCs were isolated from 4 healthy donors and they were inducted to differentiate until 14 days in both hPL and SF tenogenic media (hPL-TENO and SF-TENO). Cell viability and immunophenotype profile were analysed to evaluate mesenchymal stem cell (MSC) characteristics in both xenogenic-free media. Moreover, the expression of stemness and tendon-related markers upon cell differentiation by RT-PCR, protein staining and cytofluorimetric analysis were also performed. Our results showed the two xenogenic-free media well support cell viability of ASCs and maintain their MSC nature as demonstrated by their typical immunophenototype profile and by the expression of NANOG, OCT4 and Ki67 genes. Moreover, both hPL-TENO and SF-TENO expressed significant high levels of

the tendon-related genes SCX, COL1A1, COL3A1, COMP, MMP3 and MMP13 already at early time points in comparison to the respective controls. Significant up-regulations in scleraxis, collagen and tenomodulin proteins were also demonstrated at in both differentiated SF and hPL ASCs. In conclusion, we demonstrated firstly the feasibility of both serum and xenogenic-free media tested to culture ASCs moving forward the GMP-compliant approaches for clinical scale expansion of human MSCs needed for therapeutical application of stem cells. Moreover, a combination of CTGF, BMP-12, TGFβ3 and AA factors strongly and rapidly induce human ASCs to differentiate into tenocyte-like cells.

ChemistryOpen. 2019 Jan 23;8(2):155-165. doi: 10.1002/open.201800261. eCollection 2019 Feb.

Manganese-Zinc Ferrites: Safe and Efficient Nanolabels for Cell Imaging and Tracking In Vivo.

Herynek V[1,2], Turnovcová K[3], Gálisová A[1], Kaman O[4], Mareková D[3], Koktan J[4,5], Vosmanská M[5], Kosinová L[6], Jendelová P[3].
Author information

Abstract

Manganese-zinc ferrite nanoparticles were synthesized by using a hydrothermal treatment, coated with silica, and then tested as efficient cellular labels for cell tracking, using magnetic resonance imaging (MRI) in vivo. A toxicity study was performed on rat mesenchymal stem cells and C6 glioblastoma cells. Adverse effects on viability and cell proliferation were observed at the highest concentration (0.55 mM) only; cell viability was not compromised at lower concentrations. Nanoparticle internalization was confirmed by transmission electron microscopy. The particles were found in membranous vesicles inside the cytoplasm. Although the metal content (0.42 pg Fe/cell) was lower compared to commercially available iron oxide nanoparticles, labeled cells reached a comparable relaxation rate R_2, owing to higher nanoparticle relaxivity. Cells from transgenic luciferase-positive rats were used for in vivo experiments. Labeled cells were transplanted into the muscles of non-bioluminescent rats and visualized by MRI. The cells produced a distinct hypointense signal in T_2- or T_2*-weighted MR images in vivo. Cell viability in vivo was verified by bioluminescence.

Regen Biomater. 2019 Feb;6(1):49-59. doi: 10.1093/rb/rby025. Epub 2018 Dec 22.

Dental pulp stem cells and Bonelike® for bone regeneration in ovine model.

Campos JM[1,2,3], Sousa AC[4,5], Caseiro AR[1,2,4], Pedrosa SS[1,2], Pinto PO[1,2,3], Branquinho MV[1,2], Amorim I[6,7,8], Santos JD[4,5], Pereira T[1,2], Mendonça CM[1,2], Afonso A[9], Atayde LM[1,2], Maurício AC[1,2].
Author information

Abstract

Development of synthetic bone substitutes has arisen as a major research interest in the need to find an alternative to autologous bone grafts. Using an ovine model, the present pre-clinical study presents a synthetic bone graft (Bonelike®) in combination with a cellular system as an alternative for the regeneration of non-critical defects. The association of biomaterials and cell-based therapies is a promising strategy for bone tissue engineering. Mesenchymal stem cells (MSCs) from human dental

pulp have demonstrated both *in vitro* and *in vivo* to interact with diverse biomaterial systems and promote mineral deposition, aiming at the reconstruction of osseous defects. Moreover, these cells can be found and isolated from many species. Non-critical bone defects were treated with Bonelike® with or without MSCs obtained from the human dental pulp. Results showed that Bonelike® and MSCs treated defects showed improved bone regeneration compared with the defects treated with Bonelike® alone. Also, it was observed that the biomaterial matrix was reabsorbed and gradually replaced by new bone during the healing process. We therefore propose this combination as an efficient binomial strategy that promotes bone growth and vascularization in non-critical bone defects.

Biotechnol Bioeng. 2019 Feb 10. doi: 10.1002/bit.26950. [Epub ahead of print]

Generation and characterization of a functional human adipose-derived multipotent mesenchymal stromal cell line.

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Author information

Abstract

Multipotent mesenchymal stromal cells (MSC) and MSC-derived products have emerged as promising therapeutic tools. To fully exploit their potential, further mechanistic studies are still necessary and bioprocessing needs to be optimized, which requires an abundant supply of functional MSC for basic research. To address this need, here we used a novel technology to establish a human adipose-derived MSC line with functional characteristics representative of primary MSC. Primary MSC were isolated and subjected to lentiviral transduction with a library of expansion genes. Clonal cell lines were generated and evaluated based on their morphology, immunophenotype and proliferation potential. One clone (K5 iMSC) was then selected for further characterization. This clone had integrated a specific transgene combination including genes involved in stemness and maintenance of adult stem cells. Favourably, the K5 iMSC showed cell characteristics resembling juvenile MSC, as they displayed a shorter cell length and enhanced migration and proliferation compared to the non-immortalized original primary MSC ($p<0.05$). Still, their immunophenotype and differentiation potential corresponded to the original primary MSC and the MSC definition criteria, and cytogenetic analyses revealed no clonal aberrations. We conclude that the technology used is applicable to generate functional MSC lines for basic research and possible future bioprocessing applications.

Spectrochim Acta A Mol Biomol Spectrosc. 2019 Jan 29;213:384-390. doi: 10.1016/j.saa.2019.01.069. [Epub ahead of print]

Probing primary mesenchymal stem cells differentiation status by micro-Raman spectroscopy.

Lazarević JJ[1], Kukolj T[2], Bugarski D[2], Lazarević N[3], Bugarski B[4], Popović ZV[5].
Author information

Abstract

We have employed micro-Raman spectroscopy to get insight into intrinsic biomolecular profile of individual mesenchymal stem cell isolated from periodontal ligament. Furthermore, these cells were stimulated towards adipogenic, chondrogenic, and osteogenic lineages and their status of differentiation was assessed using micro-Raman spectroscopy. In both cases, glass coverslips were used as substrates, due to their wide availability and cost effectiveness. In all sample groups, the same type of behavior was observed, manifested as changes in Raman spectra: the increase of relative intensity of protein/lipid bands and decrease of nucleic acid bands. Comprehensive statistical analysis in the form of principal component analysis was performed, which revealed noticeable grouping of cells with the similar features. Despite the inhomogeneity of primary stem cells and their differentiated lineages, we demonstrated that micro-Raman spectroscopy is sufficient for distinguishing cells' status, which can be valuable for medical and clinical application.

Vet Immunol Immunopathol. 2019 Feb;208:6-15. doi: 10.1016/j.vetimm.2018.12.003. Epub 2018 Dec 18.

Comparative analysis and characterization of soluble factors and exosomes from cultured adipose tissue and bone marrow mesenchymal stem cells in canine species.

Villatoro AJ[1], Alcoholado C[2], Martín-Astorga MC[3], Fernández V[1], Cifuentes M[3], Becerra J[4].
Author information

Abstract

The two main sources of mesenchymal stem cell (MSCs) in the canine species are bone marrow (cBM-MSCs) and adipose tissue (cAd-MSCs). The secretion of multitude bioactive molecules, included under the concept of secretome and found in the cultured medium, play a predominant role in the mechanism of action of these cells on tissue regeneration. Although certain features of its characterization are well documented, their secretory profiles remain unknown. We described and compared, for the first time, the secretory profile and exosomes characterization in standard monolayer culture of MSCs from both sources of the same donor as well as its immunomodulatory potential. We found that despite the similarity in surface immunophenotyping and trilineage differentiation, there are several differences in terms of proliferation rate and secretory profile. cAd-MSCs have advantages in proliferative capacity, whereas cBM-MSCs showed a significantly higher secretory production of some soluble factors (IL-10, IL-2, IL-6, IL-8, IL-12p40, IFN-γ, VEGF-A, NGF-β, TGF-β, NO and PGE2) and exosomes under the same standard culture conditions. Proteomics analysis confirm that cBM-MSCs exosomes have a greater number of characterized proteins involved in metabolic processes and in the regulation of biological processes compared to cAd-MSCs. On the other hand, secretome from both sources demonstrate similar immunomodulatory capacity when tested in mitogen stimulated lymphocyte reaction, but not in their exosomes at the dose used. Considering that the use of secretome open as a new therapeutic strategy for different diseases, without the need to implant cells, those biological differences should be considered, when choosing the MSCs source, for either cellular implantation or direct use of secretome for a specific clinical application