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Mesenchymal stem cell homing towards cancer cells is increased by enzyme activity of cathepsin D.

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Author information

Abstract

Mesenchymal stem cells home towards inflammatory microenvironments, such as the tumour stroma, where they have been shown to have both pro- and anti-tumorigenic effects. Here, we demonstrate that the aspartic acid protease cathepsin D is part of the chemoattraction process. Using a Boyden chamber co-culture system, the migration of the mesenchymal stem cells and their invasion through Matrigel increased in the presence of breast cancer MDA-MB-231 cells, colon cancer HT29 cells or their conditioned media. Mesenchymal stem cell movement was reduced by protease inhibitors of matrix metalloproteinases and by pepstatin A, an inhibitor of cathepsin D. We confirmed a role for cathepsin D through addition of recombinant protein, upregulation of cathepsin D release using chloroquine and knockdown of cathepsin D expression. While all cell types expressed active cathepsin D, enzymatically inactive precursor procathepsin D was expressed only at low levels by mesenchymal stem cells. Expression in mesenchymal stem cells was increased following co-culture with cancer cells. The chemoattractive effect of cathepsin required its enzymatic activity, but not changes in mesenchymal stem cell proliferation or adhesion rates. In conclusion, cathepsin D and its precursors enhance mesenchymal stem cell homing towards tumour sites, most likely by enzymatic mechanisms.

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Graphene oxide: a growth factor delivery carrier to enhance chondrogenic differentiation of human mesenchymal stem cells in 3D hydrogels.

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Abstract

Cartilage engineering with stem cells in 3D scaffolds is a promising future therapy to treat cartilage defects. One challenge in the field is to design carriers to efficaciously deliver biological factors in 3D scaffolds containing stem cells to appropriately guide differentiation of these cells in same scaffolds and promote specific tissue synthesis. Graphene-based 2D nanomaterials have recently attracted extensive interest for their biomedical applications as they can adsorb a plethora of biological molecules, thus offering high potential as delivery carriers. This study utilized graphene oxide (GO) flakes to adsorb

transforming growth factor β3 (TGF-β3), which were then incorporated into a collagen hydrogel. Human mesenchymal stem cells (hMSCs) were encapsulated in the same gel and chondrogenic differentiation assessed. The study showed GO flakes adsorbed >99 % TGF-β3 with <1.7 % release. Adsorbed TGF-β3 retained a similar conformation to its dissolved counterpart (free protein) but importantly demonstrated greater conformational stability. Smad2 phosphorylation was promoted, and higher chondrogenic gene expression and cartilage-specific extracellular matrix deposition were achieved compared to exogenously delivering TGF-β3 in culture media. Effects were sustained in long-term 28-day culture. The results demonstrate GO flakes as highly-efficient for delivering GFs in 3D to guide cells in the same scaffold and induce tissue formation. The ability of GO flakes to provide sustained local delivery makes this material attractive for tissue engineering strategies, in particular for regionally-specific MSC differentiation (e.g. osteochondral tissue engineering). STATEMENT OF SIGNIFICANCE: Cartilage engineering involving stem cells in 3D scaffolds is a promising future therapy to treat cartilage defects which can lead to debilitating conditions such as osteoarthritis. However, this field faces the challenge to design delivery carriers to efficaciously deliver biological factors inside these 3D cell-containing scaffolds for appropriately-guided cell differentiation. Graphene-based 2D nanomaterials offer high potential as delivery carriers, but to date studies using them to deliver biological factors have been restricted to 2D substrates, non-scaffold cell masses, or acellular 3D scaffolds. Our study for the first time demonstrated simultaneously incorporating both human mesenchymal stem cells (hMSCs) and GO (graphene oxide)-adsorbed growth factor TGFβ3 into a 3D scaffold, where GO-adsorbed TGFβ3 enhanced chondrogenic differentiation of hMSCs and cartilage-tissue synthesis throughout the scaffold without needing to repeatedly supply TGFβ3 exogenously.

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Aligned fibrous decellularized cell derived matrices for mesenchymal stem cell amplification.

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Abstract

Biochemical and biophysical stimuli of stem cell niches finely regulate the self-renewal/differentiation equilibrium. Replicating this in vitro is technically challenging, making the control of stem cell functions difficult. Cell derived matrices capture certain aspect of niches that influence fate decisions. Here aligned fibrous matrices synthesized by MC3T3 cells are produced and the role of matrix orientation and stiffness on the maintenance of stem cell characteristics and adipo- or osteo-genic differentiation of murine mesenchymal stem cells (mMSCs) is investigated. Decellularized matrices promoted mMSC proliferation. Fibrillar alignment and matrix stiffness work in concert in defining cell fate. Soft matrices preserve stemness, whereas stiff ones, in presence of biochemical supplements, promptly induce differentiation. Matrix alignment impacts the homogeneity of the cell population, i.e. soft aligned matrices ameliorate the spontaneous adipogenic differentiation, whereas stiff aligned matrices reduce cross-differentiation. We infer that mechanical signalling is a dominant factor in mMSC fate decision

and the matrix alignment contributes to produce a more homogeneous environment, which results in a uniform response of cells to biophysical environment. Matrix thus produced can be obtained in vitro in a facile and consistent manner and can be used for homogeneous stem cell amplification or for mechanotransduction-related studies

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Luciferase-based reporting of suicide gene activity in murine mesenchymal stem cells.

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Author information

Abstract

Due to their ease of isolation, gene modification and tumor-homing properties, mesenchymal stem cells (MSCs) are an attractive cellular vehicle for the delivery of toxic suicide genes to a variety of cancers in pre-clinical models. In addition, the incorporation of suicide genes in stem cell-derived cell replacement therapies improves their safety profile by permitting graft destruction in the event of unexpected tumorigeneses or unwanted differentiation. Due to the functional requirement of ATP for the Firefly luciferase gene Luc2 to produce light, luciferase-based reporting of cytotoxicity can be engineered into potential cell therapies. Consequently, we nucleofected mammalian expression plasmids containing both the Luc2 and the yeast fusion cytosine deaminase uracil phosphoribosyltransferase (CDUPRT) genes for expression in murine MSCs to assess luciferase as a reporter of suicide gene cytotoxicity, and MSC as vehicles of suicide gene therapy. In vitro bioluminescence imaging (BLI) showed that following the addition of the non-toxic prodrug fluorocytosine (5-FC), CDUPRT-expressing MSCs displayed enhanced cytotoxicity in comparison to Luc2 reporter MSC controls. This study demonstrates the utility of luciferase as a reporter of CDUPRT-mediated cytotoxicity in murine MSC using BLI.

Stem Cells Transl Med. 2019 Jul 16. doi: 10.1002/sctm.19-0044. [Epub ahead of print]

Concise Review: Challenges in Clinical Development of Mesenchymal Stromal/Stem Cells.

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Abstract

Identified 50 years ago, mesenchymal stromal/stem cells (MSC) immediately generated a substantial interest among the scientific community because of their differentiation plasticity and hematopoietic supportive function. Early investigations provided evidence of a relatively low engraftment rate and a transient benefit for challenging congenital and acquired diseases. The reasons for these poor therapeutic benefits forced the entire field to reconsider MSC mechanisms of action together with their ex vivo manipulation procedures. This phase resulted in advances in MSCs processing and the hypothesis that MSC-tissue supportive functions may be prevailing their differentiation plasticity, broadening the spectrum of MSCs therapeutic potential far beyond their lineage-restricted

commitments. Consequently, an increasing number of studies have been conducted for a variety of clinical indications, revealing additional challenges and suggesting that MSCs are still lagging behind for a solid clinical translation. For this reason, our aim was to dissect the current challenges in the development of still promising cell types that, after more than half a century, still need to reach their maturity

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Transplantation of autologous bone marrow-derived mesenchymal stem cells under arthroscopic surgery with microfracture versus microfracture alone for articular cartilage lesions in the knee: A multicenter prospective randomized control clinical trial.

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Author information

Abstract

INTRODUCTION:

To investigate the efficacy of the transplantation of autologous bone marrow-derived mesenchymal stem cells (BMSCs) under arthroscopy with microfracture (MFX) compared with microfracture alone.

METHODS:

Eleven patients with a symptomatic articular cartilage defect of the knee were included in the study. They were randomized to receive BMSCs with MFX (cell-T group, n=7) or MFX alone (control group, n=4). Clinical results were evaluated using International Knee Documentation committee (IKDC) knee evaluation questionnaires and the Knee Injury and Osteoarthritis Outcome Score (KOOS) before and 48 weeks after surgery. Quantitative and qualitative assessments of repair tissue were carried out at 48 weeks by T2 mapping of magnetic resonance images (MRIs) and the magnetic resonance observation of cartilage repair tissue (MOCART) scoring system with follow-up MRI.

RESULTS:

No significant differences between preoperative and postoperative IKDC and KOOS were observed in the cell-T or control group. However, forty-eight weeks after surgery, the cell-T group showed a trend for a greater KOOS QOL score compared with the control group (79.4 vs. 39.1, respectively; P=0.07). The T2 value did not differ significantly between the two groups, but the mean MOCART score was significantly higher in the cell-T group than in the control group (P=0.02).

CONCLUSIONS:

Compared with MFX alone, BMSC transplantation with MFX resulted in better postoperative healing of the cartilage and subchondral bone as determined by the MOCART score. Clinically, BMSC transplantation with MFX gave a higher KOOS QOL score after 48 weeks.

Cells. 2019 Jul 15;8(7). pii: E724. doi: 10.3390/cells8070724.

Human Platelet Lysate as a Functional Substitute for Fetal Bovine Serum in the Culture of Human Adipose Derived Stromal/Stem Cells.

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Abstract

INTRODUCTION:

Adipose derived stromal/stem cells (ASCs) hold potential as cell therapeutics for a wide range of disease states; however, many expansion protocols rely on the use of fetal bovine serum (FBS) as a cell culture nutrient supplement. The current study explores the substitution of lysates from expired human platelets (HPLs) as an FBS substitute.

METHODS:

Expired human platelets from an authorized blood center were lysed by freeze/thawing and used to examine human ASCs with respect to proliferation using hematocytometer cell counts, colony forming unit-fibroblast (CFU-F) frequency, surface immunophenotype by flow cytometry, and tri-lineage (adipocyte, chondrocyte, osteoblast) differentiation potential by histochemical staining.

RESULTS:

The proliferation assays demonstrated that HPLs supported ASC proliferation in a concentration dependent manner, reaching levels that exceeded that observed in the presence of 10% FBS. The concentration of 0.75% HPLs was equivalent to 10% FBS when utilized in cell culture media with respect to proliferation, immunophenotype, and CFU-F frequency. When added to osteogenic, adipogenic, and chondrogenic differentiation media, both supplements showed appropriate differentiation by staining.

CONCLUSION:

HPLs is an effective substitute for FBS in the culture, expansion and differentiation of human ASCs suitable for pre-clinical studies; however, additional assays and analyses will be necessary to validate HPLs for clinical applications and regulatory approval.

Cell Tissue Res. 2019 Jul 15. doi: 10.1007/s00441-019-03069-9. [Epub ahead of print]

The efficacy of different sources of mesenchymal stem cells for the treatment of knee osteoarthritis.

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Abstract

Osteoarthritis (OA) is a common cause of chronic pain and disability. Regenerative therapies using mesenchymal stem cells (MSCs) provide an option for OA treatment as it could potentially regenerate

the damaged cartilage. Bone marrow, adipose tissue and synovium are common MSC sources. The aim is to compare the therapeutic effect of MSCs from bone marrow, adipose tissue and synovium; combining its differentiation potential and accessibility, to decide the optimal source of MSCs for the treatment of knee OA. A comparison of preclinical and clinical studies using MSCs has been made with regard to treatment outcomes, isolation procedure and differentiation potential. All types of MSCs are effective at improving the clinical and structural condition of OA patients, but the longevity of the treatment, i.e. an effect that is maintained for at least 2 years, cannot be guaranteed. This review highlighted great variations in selection criteria and culture expansion conditions of MSCs between the literature and clinical trials. It also emphasised a substantial diversity and lack of consistency in the assessment mythology of clinical outcome after completion of MSC therapies procedures. A more cohesive methodology is required to evaluate the outcome of MSC treatments using quantitative and standardised frameworks in order to be able to directly compare results. Larger population of patients are recommended to assess the quality of MSC when designing studies and clinical trials to reaffirm the efficacy of MSC treatment prior to and within the clinical trials and follow up studies.

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Decellularized extracellular matrix gelloids support mesenchymal stem cell growth and function in vitro.

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Abstract

Volumetric muscle loss (VML) injuries are irrecoverable due to a significant loss of regenerative elements, persistent inflammation, extensive fibrosis, and functional impairment. When used in isolation, previous stem cell and biomaterial-based therapies have failed to regenerate skeletal muscle at clinically relevant levels. The extracellular matrix (ECM) microenvironment is crucial for the viability, stemness, and differentiation of stem cells. Decellularized-ECM (D-ECM) scaffolds are at the forefront of ongoing research to develop a viable therapy for VML. Due to the retention of key ECM components, D-ECM scaffolds provide an excellent substrate for the adhesion and migration of several cell types. Mesenchymal stem cells (MSCs) possess regenerative and immunomodulatory properties, and are currently under investigation in clinical trials for a wide range of medical conditions. However, a major limitation to the use of MSCs in clinical applications is their poor viability at the site of transplantation. In this study, we have fabricated spherical scaffolds composed of gelatin and skeletal muscle D-ECM for the adhesion and delivery of MSCs to the site of VML injury. These spherical scaffolds termed 'gelloids' supported MSC survival, expansion, trophic factor secretion, immunomodulation, and myogenic protein expression in vitro. Future studies would determine the therapeutic efficacy of this approach in a murine model of VML injury.

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Mesenchymal stromal cells contract collagen more efficiently than dermal fibroblasts: Implications for cytotherapy.

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Author information

Abstract

BACKGROUND:

Stem cell therapy is the next generation a well-established technique. Cell therapy with mesenchymal stem cells (MSC) has been demonstrated to enhance wound healing in diabetic mice, at least partly due to improved growth factor production. However, it is unclear whether MSC can biomechanically affect wound closure. Utilizing the well-established cell-populated collagen gel contraction model we investigated the interactions between MSC and the extracellular matrix.

METHODS:

Murine fetal liver-derived Mesenchymal Stem Cells (MSCs) or fetal Dermal Fibroblasts (DFs) were cultured in cell-populated collagen gels (CPCGs). The effect of cell density, conditioned media, growth factors (TGF-B1, FGF, PDGF-BB), cytoskeletal disruptors (colchicine, cytochalasin-D), and relative hypoxia on gel contraction were evaluated. Finally, we also measured the expression of integrin receptors and some growth factors by MSCs within the contracting gels.

RESULTS:

Our results show that at different densities, MSCs induced a higher gel contraction compared to DFs. Higher cell density resulted in faster and more complete contraction of CPCGs. Cytoskeletal inhibitors either inhibited or prevented MSC-mediated contraction in a dose dependent fashion. Growth factors, conditioned media from both MSC and DF, and hypoxia all influenced CPCG contraction.

DISCUSSION:

The results suggest that MSCs are capable of directly contributing to wound closure through matrix contraction, and they are more effective than DF. In addition, this study demonstrates the importance of how other factors such as cell concentration, cytokines, and oxygen tension can provide potential modulation of therapies to correct wound healing impairments

Biochimie. 2019 Jul 11;165:76-89. doi: 10.1016/j.biochi.2019.07.009. [Epub ahead of print]

The relationship between molecular content of mesenchymal stem cells derived exosomes and their potentials: Opening the way for exosomes based therapeutics.

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Author information

Abstract

At least, more than half of our understanding of extracellular vesicles owes to the studies conducted over the past few years. When it became clear that the exosomes have various potentials in medicine, extensive research has focused on these potentials in a variety of areas including cancer, drug delivery and regenerative medicine. The growing understanding of molecular structure and functions of exosomes causes the vision to become brighter in the exosomes complexity, and our attitude toward these vesicles has undergone changes accordingly. Proteomic and transcriptomic studies on exosomes have highlighted their molecular diversity. In this review, we explicitly examine the exosomes composition, molecular structure and their therapeutic potentials in some diseases. Due to the very heterogeneous nature of exosomes, the process of their use as a therapeutic agent in the clinic has been challenged. We are still at the beginning of recognizing the molecular composition of exosomes and mechanisms that affect their physiology and biology. The growing trend of engineering of exosomes has shown a promising future to further utilize them in a different field. Molecular profiling of exosomes and their content for their related potentials in regenerative medicine should be done exactly for further defining a minimum content for specific therapeutic potentials.

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The Applications of 3D Printing for Craniofacial Tissue Engineering.

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Abstract

Three-dimensional (3D) printing is an emerging technology in the field of dentistry. It uses a layer-by-layer manufacturing technique to create scaffolds that can be used for dental tissue engineering applications. While several 3D printing methodologies exist, such as selective laser sintering or fused deposition modeling, this paper will review the applications of 3D printing for craniofacial tissue engineering; in particular for the periodontal complex, dental pulp, alveolar bone, and cartilage. For the periodontal complex, a 3D printed scaffold was attempted to treat a periodontal defect; for dental pulp, hydrogels were created that can support an odontoblastic cell line; for bone and cartilage, a polycaprolactone scaffold with microspheres induced the formation of multiphase fibrocartilaginous tissues. While the current research highlights the development and potential of 3D printing, more research is required to fully understand this technology and for its incorporation into the dental field.