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Rapid identification of genome-edited mesenchymal stem cell colonies via Cas9.

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Author information

Abstract

Mesenchymal stem cells (MSCs) have been intensively investigated and widely applied in regenerative medicine and immune modulation. However, their efficacy declines during the aging or disease process. Thus, genome-edited MSCs with over-expression or inhibition of specific genes hold a great deal of promise in terms of their therapeutic application. Here we optimized the direct PCR approach for rapid identification of genome-edited MSCs with only ten cells required, which reduces the time and labor to expand the MSC colonies. Combined with our previously optimized guide RNA structure and plasmid construction strategy for Cas9, we successfully identified MSC colonies over-expressing IL-10 in the AAVS1 locus.

Eur J Hum Genet. 2019 Mar 27. doi: 10.1038/s41431-019-0387-4. [Epub ahead of print]

Stakeholder views and attitudes towards prenatal and postnatal transplantation of fetal mesenchymal stem cells to treat Osteogenesis Imperfecta.

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Abstract

The Boost Brittle Bones Before Birth (BOOSTB4) clinical trial is investigating the safety and efficacy of transplanting fetal derived mesenchymal stromal cells (MSCs) prenatally and/or in early postnatal life to treat severe Osteogenesis Imperfecta (OI). This study aimed to explore stakeholder views to understand perceived benefits or concerns, identify ethical issues and establish protocols for support and counselling. Semi-structured qualitative interviews were conducted with three groups; 1. Adults affected with OI, with and without children, and parents of children affected with OI; 2. Health professionals who work with patients with OI; 3. Patient advocates from relevant patient support groups. Interviews were digitally recorded, transcribed verbatim and analysed using thematic analysis. Interviews with 56 participants revealed generally positive views towards using fetal MSC transplantation to treat OI. Early treatment was considered advantageous for preventing fractures and reducing severity and could bring psychological benefits for parents. Common concerns were procedure safety, short/long-term side effects and whether transplantation would be effective. Difficulties inherent in decision-making were frequently discussed, as treatment efficacy is unknown and, by necessity, parents will make decisions at a time when they are vulnerable. Support needs may differ where there is a family history of OI compared to an unexpected diagnosis of OI. Explaining fetal

MSC transplantation in a way that all parents can understand, clear expectation setting, psychological support and time for reflection during the decision-making process will be crucial to allow parents to make informed decisions about participation in the BOOSTB4 clinical trial.

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Development of a microdevice-based human mesenchymal stem cell-mediated drug delivery system.

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Abstract

Cell-mediated drug delivery systems utilize living cells as vehicles to achieve controlled delivery of drugs. One of the systems features integrating cells with disk-shaped microparticles termed microdevices into cell-microdevice complexes that possess some unique advantages over their counterparts. Human mesenchymal stem cells (hMSCs) have been extensively studied as therapeutic cells and used as carrier cells for drug-loaded nanoparticles or other functional nanoparticles. This article presents the development of a microdevice-based hMSC-mediated drug delivery system for the first time. This study revealed that the microdevices could be attached to the hMSCs in a controlled and versatile manner; the produced hMSC-microdevice complexes were stable over cultivation and trypsinization, and the microdevice attachment did not affect the viability and proliferation of the hMSCs. Moreover, cultured microdevice-bound hMSCs retained their abilities to migrate on a flat surface, form a spheroid, and actively dissociate from the spheroid. These results indicate that this microdevice-based hMSC-mediated system promises to be further developed into a clinically viable drug delivery system.

Bone Joint Res. 2019 Mar 2;8(2):101-106. doi: 10.1302/2046-3758.82.BJR-2018-0134.R1. eCollection 2019 Feb.

Patient-specific meniscus prototype based on 3D bioprinting of human cell-laden scaffold.

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Abstract

OBJECTIVES:

Meniscal injuries are often associated with an active lifestyle. The damage of meniscal tissue puts young patients at higher risk of undergoing meniscal surgery and, therefore, at higher risk of osteoarthritis. In this study, we undertook proof-of-concept research to develop a cellularized human meniscus by using 3D bioprinting technology.

METHODS:

A 3D model of bioengineered medial meniscus tissue was created, based on MRI scans of a human volunteer. The Digital Imaging and Communications in Medicine (DICOM) data from these MRI scans were processed using dedicated software, in order to obtain an STL model of the structure. The chosen

3D Discovery printing tool was a microvalve-based inkjet printhead. Primary mesenchymal stem cells (MSCs) were isolated from bone marrow and embedded in a collagen-based bio-ink before printing. LIVE/DEAD assay was performed on realized cell-laden constructs carrying MSCs in order to evaluate cell distribution and viability.

RESULTS:

This study involved the realization of a human cell-laden collagen meniscus using 3D bioprinting. The meniscus prototype showed the biological potential of this technology to provide an anatomically shaped, patient-specific construct with viable cells on a biocompatible material.

CONCLUSION:

This paper reports the preliminary findings of the production of a custom-made, cell-laden, collagen-based human meniscus. The prototype described could act as the starting point for future developments of this collagen-based, tissue-engineered structure, which could aid the optimization of implants designed to replace damaged menisci.

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Long-Lasting Anti-Inflammatory Activity of Human Microfragmented Adipose Tissue.

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Abstract

Over the last few years, human microfragmented adipose tissue (MFAT), containing significant levels of mesenchymal stromal cells (MSCs) and obtained from fat lipoaspirate (LP) through a minimal manipulation in a closed system device, has been successfully used in aesthetic medicine as well as in orthopedic and general surgery. Interestingly, in orthopedic diseases, this ready-to-use adipose tissue cell derivative seems to have a prolonged time efficacy even upon a single shot injection into osteoarthritic tissues. Here, we investigated the long-term survival and content of MSCs as well the anti-inflammatory activity of LP and its derived MFAT in vitro, with the aim to better understand a possible in vivo mechanism of action. MFAT and LP specimens from 17 human donors were investigated side by side. During a long-term culture in serum-free medium, we found that the total cell number as well the MSC content in MFAT decreased more slowly if compared to those from LP specimens. The analysis of cytokines and growth factors secreted into the conditioned medium (CM) was similar in MFAT and LP during the first week of culture, but the total amount of cytokines secreted by LP decreased much more rapidly than those produced by MFAT during prolonged culture (up to 28 days). Similarly, the addition of MFAT-CM recovered at early (3-7 days) and late stage (14-28 days) of culture strongly inhibited inflammatory function of U937 monocyte cell line, whereas the anti-inflammatory activity of LP-CM was drastically reduced after only 7 days of culture. We conclude that MFAT is an effective preparation with a long-lasting anti-inflammatory activity probably mediated by a

long-term survival of their MSC content that releases a combination of cytokines that affect several mechanisms involved in inflammation processes

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Comparison of Immunosuppressive and Angiogenic Properties of Human Amnion-Derived Mesenchymal Stem Cells between 2D and 3D Culture Systems.

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Abstract

The secretion of potential therapeutic factors by mesenchymal stem cells (MSCs) has aroused much interest given the benefits that it can bring in the field of regenerative medicine. Indeed, the *in vitro* multipotency of these cells and the secretive capacity of both angiogenic and immunomodulatory factors suggest a role in tissue repair and regeneration. However, during culture, MSCs rapidly lose the expression of key transcription factors associated with multipotency and self-renewal, as well as the ability to produce functional paracrine factors. In our study, we show that a three-dimensional (3D) culture method is effective to induce MSC spheroid formation, to maintain the multipotency and to improve the paracrine activity of a specific population of human amnion-derived MSCs (hAMSCs). The regenerative potential of both 3D culture-derived conditioned medium (3D CM) and their exosomes (EXO) was assessed against 2D culture products. In particular, tubulogenesis assays revealed increased capillary maturation in the presence of 3D CM compared with both 2D CM and 2D EXO. Furthermore, 3D CM had a greater effect on inhibition of PBMC proliferation than both 2D CM and 2D EXO. To support this data, hAMSC spheroids kept in our 3D culture system remained viable and multipotent and secreted considerable amounts of both angiogenic and immunosuppressive factors, which were detected at lower levels in 2D cultures. This work reveals the placenta as an important source of MSCs that can be used for eventual clinical applications as cell-free therapies.

J Clin Med. 2019 Mar 21;8(3). pii: E392. doi: 10.3390/jcm8030392.

Autologous Matrix-Induced Chondrogenesis (AMIC) and AMIC Enhanced by Autologous Concentrated Bone Marrow Aspirate (BMAC) Allow for Stable Clinical and Functional Improvements at up to 9 Years Follow-Up: Results from a Randomized Controlled Study.

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Abstract

The aims of the study were to evaluate long-term outcomes after autologous matrix-induced chondrogenesis (AMIC) in the treatment of focal chondral lesions and to assess the possible improvements given by the combination of this technique with bone marrow aspirate concentrate (BMAC). Twenty-four patients (age range 18⁻55 years) affected by focal knee chondral lesions were

treated with standard AMIC or AMIC enhanced by BMAC (AMIC+). Pain (Visual Analogue Scale (VAS)) and functional scores (Lysholm, International Knee Documentation Committee (IKDC), Tegner, Knee injury and Osteoarthritis Outcome Score (KOOS)) were collected pre-operatively and then at 6, 12, 24, 60, and 100 months after treatment. Magnetic resonance imaging (MRI) evaluation was performed pre-operatively and at 6, 12, and 24 months follow-ups. Patients treated with AMIC+ showed higher Lysholm scores (p = 0.015) and lower VAS (p = 0.011) in comparison with patients in the standard AMIC group at the 12 months follow-up. Both treatments allowed for functional and pain improvements with respect to pre-operative levels lasting up to 100 months. MRI revealed consistent cartilage repair at 24 months in both groups. This study shows that AMIC and AMIC+ are effective treatments for focal chondral lesions with beneficial effect lasting up to 9 years. AMIC+ allows for faster recovery from injury, and is thus more indicated for patients requiring a prompt return to activity. Level of evidence: II, randomized controlled trial in an explorative cohort.