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Stem cells out of the bag: characterization of *ex vivo* expanded mesenchymal stromal cells for possible clinical use.

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Abstract

AIM:

Mesenchymal stromal cells (MSC) are a promising tool for cellular therapy and regenerative medicine. One major difficulty in establishing a MSC expansion protocol is the large volume of bone marrow (BM) required. We studied whether cells trapped within a collection bag and filter system could be considered as a source of MSC.

RESULTS:

From the 20 BM collection bag and filter systems, we recovered an average of 1.68×10^8 mononuclear cells, which is the equivalent to 60 ml of filtered BM. Mononuclear cells were expanded *ex vivo* to 17×10^6 MSC, with purity shown by a $CD44^+$, $CD105^+$, $CD90^+$ and $CD73^+$ immunophenotype, a reduction of 20% proliferating cells in a mixed lymphocyte reaction and also the ability of adipocyte differentiation.

CONCLUSION:

Long-term MSC cultures were established from the usually discarded BM collection bag and filter, maintaining an appropriate phenotype and function, being suitable for both investigation and clinical settings.

Stem Cell Res Ther. 2020 Mar 4;11(1):99. doi: 10.1186/s13287-020-01611-z.

Priming with inflammatory cytokines is not a prerequisite to increase immune-suppressive effects and responsiveness of equine amniotic mesenchymal stromal cells.

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Abstract

BACKGROUND:

Equine amniotic mesenchymal stromal cells (AMSCs) and their conditioned medium (CM) were evaluated for their ability to inhibit in vitro proliferation of peripheral blood mononuclear cells (PBMCs) with and without priming. Additionally, AMSC immunogenicity was assessed by expression of MHCI and MHCII and their ability to counteract the in vitro inflammatory process.

METHODS:

Horse PBMC proliferation was induced with phytohemagglutinin. AMSC priming was performed with 10 ng/ml of TNF-α, 100 ng/ml of IFN-γ, and a combination of 5 ng/ml of TNF-α and 50 ng/ml of IFN-γ. The CM generated from naïve unprimed and primed AMSCs was also tested to evaluate its effects on equine endometrial cells in an in vitro inflammatory model induced by LPS. Immunogenicity marker expression (MHCI and II) was evaluated by qRT-PCR and by flow cytometry.

RESULTS:

Priming does not increase MHCI and II expression. Furthermore, the inhibition of PBMC proliferation was comparable between naïve and conditioned cells, with the exception of AMSCs primed with both TNF-α and IFN-γ that had a reduced capacity to inhibit T cell proliferation. However, AMSC viability was lower after priming than under other experimental conditions. CM from naïve and primed AMSCs strongly inhibited PBMC proliferation and counteracted the inflammatory process, rescuing about 65% of endometrial cells treated by LPS.

CONCLUSION:

AMSCs and their CM have a strong capacity to inhibit PBMC proliferation, and priming is not necessary to improve their immunosuppressive activity or reactivity in an inflammatory in vitro model.

Eur Cell Mater. 2020 Mar 3;39:156-170. doi: 10.22203/eCM.v039a10.

Intervertebral disc and endplate cell characterisation highlights annulus fibrosus cells as the most promising for tissue-specific disc degeneration therapy.

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Abstract

Degenerative processes of the intervertebral disc (IVD) and cartilaginous endplate lead to chronic spine pathologies. Several studies speculated on the intrinsic regenerative capacity of degenerated IVD related to the presence of local mesenchymal progenitors. However, a complete characterisation of the resident IVD cell populations, particularly that isolated from the endplate, is lacking. The purpose of the present study was to characterise the gene expression profiles of human nucleus pulposus (NPCs), annulus fibrosus (AFCs) and endplate (EPCs) cells, setting the basis for future studies aimed at identifying the most promising cells for regenerative purposes. Cells isolated from NP, AF and EP were analysed after in vitro expansion for their stemness ability, immunophenotype and gene profiles by large-scale microarray analysis. The three cell populations shared a similar clonogenic, adipogenic and osteogenic potential, as well as an immunophenotype with a pattern resembling that of mesenchymal stem cells. NPCs maintained the greatest chondrogenic potential and shared with EPCs the loss of proliferation capability during expansion. The largest number of selectively highly expressed stemness, chondrogenic/tissue-specific and surface genes was found in AFCs, thus representing the most promising source of tissue-specific expanded cells for the treatment of IVD degeneration.

Nanomaterials (Basel). 2020 Feb 29;10(3). pii: E432. doi: 10.3390/nano10030432.

Mineral-Doped Poly(L-lactide) Acid Scaffolds Enriched with Exosomes Improve Osteogenic Commitment of Human Adipose-Derived Mesenchymal Stem Cells.

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Abstract

Exosomes derived from mesenchymal stem cells are extracellular vesicles released to facilitate cell communication and function. Recently, polylactic acid (PLA), calcium silicates (CaSi), and dicalcium phosphate dihydrate (DCPD) have been used to produce bioresorbable functional mineral-doped porous scaffolds-through thermally induced phase separation technique, as materials for bone regeneration. The aim of this study was to investigate the effect of mineral-doped PLA-based porous scaffolds enriched with exosome vesicles (EVs) on osteogenic commitment of human adipose mesenchymal stem cells (hAD-MSCs). Two different mineral-doped scaffolds were produced: PLA-10CaSi-10DCPD and PLA-5CaSi-5DCPD. Scaffolds surface micromorphology was investigated by ESEM-EDX before and after 28 days immersion in simulated body fluid (HBSS). Exosomes were deposited on the surface of the scaffolds and the effect of exosome-enriched scaffolds on osteogenic commitment of hAD-MSCs cultured in proximity of the scaffolds has been evaluated by real time PCR. In addition, the biocompatibility was evaluated by direct-contact seeding hAD-MSCs on scaffolds surface-using MTT viability test. In both formulations, ESEM showed pores similar in shape (circular and elliptic) and size (from 10-30 μm diameter). The porosity of the scaffolds decreased after 28 days immersion in simulated body fluid. Mineral-doped scaffolds showed a dynamic surface and created a suitable bone-forming microenvironment. The presence of the mineral fillers increased the osteogenic commitment of hAD-MSCs. Exosomes were easily entrapped on the surface of the scaffolds and their presence improved gene expression of major markers of osteogenesis such as collagen type I, osteopontin, osteonectin, osteocalcin. The experimental scaffolds enriched with exosomes, in particular PLA-10CaSi-10DCPD, increased the osteogenic commitment of MSCs. In conclusion, the enrichment of bioresorbable functional scaffolds with exosomes is confirmed as a potential strategy to improve bone regeneration procedures.

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Extended Treatment With Mesenchymal Stromal Cells-Frankfurt am Main (MSC-FFM, Obnitix) in a Pediatric Patient With Steroid-refractory Acute Gastrointestinal Graft-Versus-Host Disease (GVHD): Case Report and Review of the Literature.

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Abstract

In acute graft-versus-host disease (aGVHD) following allogeneic hematopoietic stem cell transplantation, there are various options available after the failure of initial steroid therapy. Since the publication of the first study in 2008, mesenchymal stromal cells (MSCs) have also been used with increasing frequency, including in pediatric patients with steroid-refractory aGVHD, and the manufacturing process has undergone further development. MSC-Frankfurt am Main (MSC-FFM, Obnitix), which is manufactured from pooled mononuclear bone marrow cells from 8 donors using a standardized process, resulted in a response rate of 84% in children with steroid-refractory aGVHD. We report on a 13-year-old female patient with acute myeloid leukemia who received Obnitix as a third-line treatment for gastrointestinal (GI) aGVHD in a life-threatening situation. The patient was initially given a total of 4 Obnitix infusions as per the regulatory approval, with her symptoms improving from day 9 after the first infusion. The second cycle of 4 Obnitix infusions followed due to persistent severe protein-losing enteropathy and resulted in complete remission. A systematic review of the literature on MSC in pediatric patients with steroid-refractory aGVHD confirms that MSC treatment beyond 4 weeks is employed in accordance with treatment protocols or on a case-by-case basis. To summarize, aGVHD activity can be checked endoscopically in patients with persistent GI symptoms and a second Obnitix cycle can then be administered if appropriate, with the goal of achieving complete remission. Future studies should also investigate the potential influence of tissue repair properties as an element in MSCs' efficacy in GI aGVHD.

Physiol Res. 2019 Dec 30;68(Suppl 4):S385-S388.

Isolating stem cells from skin: designing a novel highly efficient non-enzymatic approach.

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Abstract

Stem cells are undifferentiated elements capable to acquire a specific cellular phenotype under the influence of specific stimuli, thus being involved in tissue integrity and maintenance. In the skin tissue self-renewal and wound healing after injury is a complex process, especially in adulthood, due to the aging process and the continuous exposure to damaging agents. The importance of stem cells in regenerative medicine is well known and defining or improving their isolation methods is therefore a primary and crucial step. In the present paper we present a novel method to isolate stem cells from human skin, including the involvement of a novel medium for the maintenance and expansion of in vitro cultures. The biopsies were mechanically digested and put in culture. The migrating cells were positive selected with magnetic cell sorting, characterized by flow-cytometry analysis, and viability detected by MTT assay. Cells exhibited a mesenchymal phenotype, as demonstrated by the positive acquirement of an osteogenic or adipogenic phenotype when cultured in specific conditioned media. Taken together our results disclose a novel method for culturing and expanding stem cells from skin and pave the way for future clinical applications in tissue regeneration.

Cytotherapy. 2020 Feb 26. pii: S1465-3249(20)30008-6. doi: 10.1016/j.jcyt.2020.01.006. [Epub ahead of print]

A Monte Carlo framework for managing biological variability in manufacture of autologous cell therapy from mesenchymal stromal cells therapies.

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Abstract

Manufacturing processes for autologous cell therapy need to reproducibly generate in specification (quality and quantity) clinical product. However, patient variability prevents the level of control of cell input material that could be achieved in a cell line or allogeneic-based process. We have applied literature data on bone marrow-derived mesenchymal stromal cells variability to estimate probability distributions for stem cell yields given underlying truncated normal distributions in total nucleated cell concentration, stem cell percentage and plausible aspirate volumes. Monte Carlo simulation identified potential variability in harvested stem cell number in excess of an order of magnitude. The source material variability was used to identify the proportion of donor manufacturing runs that would achieve a target yield specification of 2E7 cells in a fixed time window with given proliferative rates and different aspirate volumes. A rapid, screening, development approach was undertaken to assess culture materials and process parameters (T-flask surface, medium, feed schedule) to specify a protocol with identified proliferative rate and a consequent model-based target aspirate volume. Finally, four engineering runs of the candidate process were conducted and a range of relevant quality parameters measured including expression of markers CD105, CD73, CD44, CD45, CD34, CD11b, CD19, HLA-DR, CD146 (melanoma cell adhesion molecule), CD106 (vascular cell adhesion molecule) and SSEA-4, specific metabolic activity and vascular endothelial growth factor secretion, and osteogenic differentiation potential. Our approach of using estimated distributions from publicly available information provides a route for data-poor earl- stage developers to plan manufacture with defined risk based on rational assumptions; furthermore, the models produced by such assumptions can be used to evaluate candidate processes, and can be incrementally improved with accumulating distribution understanding or subdivision by new process variables.

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Therapeutic Potential of Mesenchymal Stem Cells for Cancer Therapy.

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Abstract

Mesenchymal stem cells (MSCs) are among the most frequently used cell type for regenerative medicine. A large number of studies have shown the beneficial effects of MSC-based therapies to treat different pathologies, including neurological disorders, cardiac ischemia, diabetes, and bone and

cartilage diseases. However, the therapeutic potential of MSCs in cancer is still controversial. While some studies indicate that MSCs may contribute to cancer pathogenesis, emerging data reported the suppressive effects of MSCs on cancer cells. Because of this reality, a sustained effort to understand when MSCs promote or suppress tumor development is needed before planning a MSC-based therapy for cancer. Herein, we provide an overview on the therapeutic application of MSCs for regenerative medicine and the processes that orchestrates tissue repair, with a special emphasis placed on cancer, including central nervous system tumors. Furthermore, we will discuss the current evidence regarding the double-edged sword of MSCs in oncological treatment and the latest advances in MSC-based anti-cancer agent delivery systems.