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Stem Cell

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Stem Cell Research Literatures (2)

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; life; research; literature

Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Abdelrazik, H., et al. (2011). "Mesenchymal stem cells expanded in human platelet lysate display a decreased inhibitory capacity on T- and NK-cell proliferation and function." Eur J Immunol **41**(11): 3281-3290.

The use of fetal bovine serum (FBS) for the culture and expansion of mesenchymal stromal cells (MSCs) limits their possible clinical applications. Although some recent studies recommended substituting FBS with human platelet lysate (HPL) for the expansion of MSCs for clinical use, the functional capacity of the expanded cells has only been partially explored. 10% FBS and two other commercial FBS-containing media (MesenCult and MesenPro) were compared with 10% HPL-containing medium for their ability to support MSCs expansion and immunomodulation. We demonstrate that HPL sustained MSC proliferation and expansion in vitro. However, the cumulative cell numbers recovered were comparable with those obtained in MesenPro medium. Moreover, we show that HPL alters the expression of some relevant MSC surface molecules, namely the DNAM-1 ligands PVR and Nectin-2, the NKG2D ligand ULBP3, the adhesion molecules CD49d and alphavbeta3 and the fibroblast-associated protein. In addition, MSCs cultured in HPL displayed impaired inhibitory capacity on T-cell proliferation to alloantigen and NK-cell proliferation and cytotoxicity. Finally, they showed decreased constitutive PGE2 production while IL-6, IL-8 and RANTES secretion were upregulated. These results imply some limitations in the use of HPL for the expansion of MSCs to be used as immunomodulators in clinical applications.

Abumaree, M. H., et al. (2017). "Human chorionic villous mesenchymal stem/stromal cells modify the effects of oxidative stress on endothelial cell functions." Placenta **59**: 74-86.

Mesenchymal stem/stromal cells derived from chorionic villi of human term placentae (pMSCs) produce a unique combination of molecules, which modulate important cellular functions of their target cells while concurrently suppressing their immune responses. These properties make MSCs advantageous candidates for cell-based therapy. Our first aim was to examine the effect of high levels of oxidative stress on pMSC functions. pMSCs were exposed to hydrogen peroxide (H2O2) and their ability to proliferate and adhere to an endothelial cell monolayer was determined. Oxidatively stressed pMSCs maintained their proliferation and adhesion potentials. The second aim was to measure the ability of pMSCs to prevent

oxidative stress-related damage to endothelial cells. Endothelial cells were exposed to H2O2, then co-cultured with pMSCs, and the effect on endothelial cell adhesion, proliferation and migration was determined. pMSCs were able to reverse the damaging effects of oxidative stress on the proliferation and migration but not on the adhesion of endothelial cells. These data indicate that pMSCs are not only inherently resistant to oxidative stress, but also protect endothelial cell functions from oxidative stress-associated damage. Therefore, pMSCs could be used as a therapeutic tool in inflammatory diseases by reducing the effects of oxidative stress on endothelial cells.

Acquistapace, A., et al. (2011). "Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer." Stem Cells **29**(5): 812-824.

Because stem cells are often found to improve repair tissue including heart without evidence of engraftment or differentiation, mechanisms underlying wound healing are still elusive. Several studies have reported that stem cells can fuse with cardiomyocytes either by permanent or partial cell fusion processes. However, the respective physiological impact of these two processes remains unknown in part because of the lack of knowledge of the resulting hybrid cells. To further characterize cell fusion, we cocultured mouse fully differentiated cardiomyocytes with human multipotent adipose-derived stem (hMADS) cells as a model of adult stem cells. We found that heterologous cell fusion promoted cardiomyocyte reprogramming back to a progenitor-like state. The resulting hybrid cells expressed early cardiac commitment and proliferation markers such as GATA-4, myocyte enhancer factor 2C, Nkx2.5, and Ki67 and exhibited a mouse genotype. Interestingly, human bone marrow-derived stem cells shared similar reprogramming properties than hMADS cells but not human fibroblasts, which suggests that these features might be common to multipotent cells. Furthermore, cardiac hybrid cells were preferentially generated by partial rather than permanent cell fusion and that intercellular structures composed of f-actin and microtubule filaments were involved in the process. Finally, we showed that stem cell mitochondria were transferred into cardiomyocytes, persisted in hybrids and were required for somatic cell reprogramming. In conclusion, by providing new insights into previously reported cell fusion processes, our data might contribute to a better understanding of stem cell-mediated regenerative mechanisms and thus, the development of more efficient stem cell-based heart therapies.

Aggarwal, S. and M. F. Pittenger (2005). "Human mesenchymal stem cells modulate allogeneic immune cell responses." Blood **105**(4): 1815-1822.

Mesenchymal stem cells (MSCs) are multipotent cells found in several adult tissues. Transplanted allogeneic MSCs can be detected in recipients at extended time points, indicating a lack of immune recognition and clearance. As well, a role for bone marrow-derived MSCs in reducing the incidence and severity of graft-versus-host disease (GVHD) during allogeneic transplantation has recently been reported; however, the mechanisms remain to be investigated. We examined the immunomodulatory functions of human MSCs (hMSCs) by coculturing them with purified subpopulations of immune cells and report here that hMSCs altered the cytokine secretion profile of dendritic cells (DCs), naive and effector T cells (T helper 1 [T(H)1] and T(H)2), and natural killer (NK) cells to induce a more anti-inflammatory or tolerant phenotype. Specifically, the hMSCs caused mature DCs type 1 (DC1) to decrease tumor necrosis factor alpha (TNF-alpha) secretion and mature DC2 to increase interleukin-10 (IL-10) secretion; hMSCs caused T(H)1 cells to decrease interferon gamma (IFN-gamma) and caused the T(H)2 cells to increase secretion of IL-4; hMSCs caused an increase in the proportion of regulatory T cells (T(Regs)) present; and hMSCs decreased secretion of IFN-gamma from the NK cells. Mechanistically, the hMSCs produced elevated prostaglandin E2 (PGE(2)) in co-cultures, and inhibitors of PGE(2) production mitigated hMSC-mediated immune modulation. These data offer insight into the interactions between allogeneic MSCs and immune cells and provide mechanisms likely involved with the in vivo MSC-mediated induction of tolerance that could be therapeutic for reduction of GVHD, rejection, and modulation of inflammation.

Ahn, J. O., et al. (2014). "Human adipose tissue-derived mesenchymal stem cells inhibit T-cell lymphoma growth in vitro and in vivo." Anticancer Res **34**(9): 4839-4847.

BACKGROUND/AIM: Human mesenchymal stem cells (hMSCs) are thought to be one of the most reliable stem cell sources for a variety of cell therapies. This study investigated the anti-tumor effect of human adipose tissue-derived mesenchymal stem cells (hAT-MSCs) on EL4 murine T-cell lymphoma in vitro and in vivo. MATERIALS AND METHODS: The growth-inhibitory effect of hAT-MSCs on EL4 tumor cells was evaluated using a WST-1 cell proliferation assay. Cell-cycle arrest and apoptosis were investigated by flow cytometry and western blot. To evaluate an anti-tumor effect of hAT-MSCs on T-cell lymphoma in vivo, CM-DiI-labeled hAT-MSCs were circumtumorally injected in tumor-bearing nude mice, and tumor size was

measured. RESULTS: hAT-MSCs inhibited T-cell lymphoma growth by altering cell-cycle progression and inducing apoptosis in vitro. hAT-MSCs inhibited tumor growth in tumor-bearing nude mice and prolonged survival time. Immunofluorescence analysis showed that hAT-MSCs migrated to tumor sites. CONCLUSION: hAT-MSCs suppress the growth of T-cell lymphoma, suggesting a therapeutic option for T-cell lymphoma.

Akimov, S., et al. (2009). "Murine bone marrow stromal cell culture with features of mesenchymal stem cells susceptible to mouse-adapted human TSE agent, Fukuoka-1." Folia Neuropathol **47**(2): 205-214.

Transmission of transmissible spongiform encephalopathies (TSEs)/prion diseases through transplantation of bone marrow (BM) has never been reported in humans. However, the use of fetal bovine serum in current protocols for generating mesenchymal stem cells (MSCs) carries the risk of iatrogenic spread. We developed a cell model from murine BM-derived MSCs and tested its susceptibility to Fukuoka-1 (Fu) strain of TSEs. The adherent cells expressed significant levels of normal prion protein, PrPC, at the time when they became immortalized. The cell culture underwent spontaneous transformation following inoculation with Fu-infected brain homogenate and became persistently infected after reinoculation with Fu agent. Extensive analysis of the original and two Fu-exposed cell cultures revealed a phenotype characteristic of MSCs with a majority of cells being positive for stem cell antigen, Sca-1. Taken together, our results demonstrate that BM-derived MSCs can be infected with TSE agents under certain conditions ex vivo. Comprehensive studies should be undertaken to address the safety of cell-based therapies in regard to iatrogenic transmission of TSEs. BM-derived cell cultures can be used for studies of molecular mechanisms underlying the cells' susceptibility to various strains of TSEs, their propagation ex vivo, and for screening of potential anti-TSEs therapeutics.

Almeida, C. R., et al. (2016). "NAP-2 Secreted by Human NK Cells Can Stimulate Mesenchymal Stem/Stromal Cell Recruitment." Stem Cell Reports **6**(4): 466-473.

Strategies for improved homing of mesenchymal stem cells (MSCs) to a place of injury are being sought and it has been shown that natural killer (NK) cells can stimulate MSC recruitment. Here, we studied the chemokines behind this recruitment. Assays were performed with bone marrow human MSCs and NK cells freshly isolated from healthy donor buffy coats. Supernatants from MSC-NK cell co-cultures can induce MSC recruitment but not to the same extent as when NK cells are present. Antibody arrays and ELISA assays confirmed that NK cells secrete RANTES (CCL5) and revealed that human NK cells secrete NAP-2 (CXCL7), a chemokine that can induce MSC migration. Inhibition with specific antagonists of CXCR2, a receptor that recognizes NAP-2, abolished NK cell-mediated MSC recruitment. This capacity of NK cells to produce chemokines that stimulate MSC recruitment points toward a role for this immune cell population in regulating tissue repair/regeneration.

Alshabibi, M. A., et al. (2017). "Mesenchymal Stem/Multipotent Stromal Cells from Human Decidua Basalis Reduce Endothelial Cell Activation." Stem Cells Dev **26**(18): 1355-1373.

Recently, we reported the isolation and characterization of mesenchymal stem cells from the decidua basalis of human placenta (DBMSCs). These cells express a unique combination of molecules involved in many important cellular functions, which make them good candidates for cell-based therapies. The endothelium is a highly specialized, metabolically active interface between blood and the underlying tissues. Inflammatory factors stimulate the endothelium to undergo a change to a proinflammatory and procoagulant state (ie, endothelial cell activation). An initial response to endothelial cell activation is monocyte adhesion. Activation typically involves increased proliferation and enhanced expression of adhesion and inflammatory markers by endothelial cells. Sustained endothelial cell activation leads to a type of damage to the body associated with inflammatory diseases, such as atherosclerosis. In this study, we examined the ability of DBMSCs to protect endothelial cells from activation through monocyte adhesion, by modulating endothelial proliferation, migration, adhesion, and inflammatory marker expression. Endothelial cells were cocultured with DBMSCs, monocytes, monocyte-pretreated with DBMSCs and DBMSC-pretreated with monocytes were also evaluated. Monocyte adhesion to endothelial cells was examined following treatment with DBMSCs. Expression of endothelial cell adhesion and inflammatory markers was also analyzed. The interaction between DBMSCs and monocytes reduced endothelial cell proliferation and monocyte adhesion to endothelial cells. In contrast, endothelial cell migration increased in response to DBMSCs and monocytes. Endothelial cell expression of adhesion and inflammatory molecules was reduced by DBMSCs and DBMSC-pretreated with monocytes. The mechanism of reduced endothelial proliferation involved enhanced phosphorylation of the tumor suppressor protein p53. Our study shows for the first time that DBMSCs protect endothelial cells from activation by inflammation triggered by monocyte adhesion and

increased endothelial cell proliferation. These events are manifest in inflammatory diseases, such as atherosclerosis. Therefore, our results suggest that DBMSCs could be usefully employed as a therapeutic strategy for atherosclerosis.

Alshatwi, A. A., et al. (2012). "Al(2)O(3) nanoparticles induce mitochondria-mediated cell death and upregulate the expression of signaling genes in human mesenchymal stem cells." J Biochem Mol Toxicol **26**(11): 469-476.

An increase in the broad usage of Al(2)O(3) nanoparticles (ANPs) in the food and agricultural sectors may produce rare hazards for human health. The objective of this study was to assess the acute toxicity of ANPs in human mesenchymal stem cells (hMSCs) in vitro. Cell viability, cellular uptake, morphology, and gene expression using quantitative real-time polymerase chain reaction (qRT-PCR) were analyzed. The results indicate that ANPs have a significant and dose-dependent effect on cytotoxicity. Control cells showed a characteristic, homogeneous nuclear staining pattern, whereas ANP-exposed cells showed abnormal nuclear morphological changes such as condensation or fragmentation. An early characteristic of apoptosis was observed in ANP-treated cells. Further confirmation of cell death in hMSCs was observed through increased expression of chosen signaling genes and also decreased expression of Bcl-2 during mitochondria-mediated cell death. Although they provide great advantages in food and agricultural products, the chronic and acute toxicity of ANPs still needs to be assessed carefully.

Altanerova, U., et al. (2017). "Human mesenchymal stem cell-derived iron oxide exosomes allow targeted ablation of tumor cells via magnetic hyperthermia." Int J Nanomedicine **12**: 7923-7936.

Magnetic hyperthermia, or the heating of tissues using magnetic materials, is a promising approach for treating cancer. We found that human mesenchymal stem cells (MSCs) isolated from various tissues and MSCs expressing the yeast cytosine deaminaseColon, two colonsuracil phosphoribosyl transferase suicide fusion gene (yCDColon, two colonsUPRT) can be labeled with Venofer, an iron oxide carbohydrate nanoparticle. Venofer labeling did not affect cell proliferation or the ability to home to tumors. All Venofer-labeled MSCs released exosomes that contained iron oxide. Furthermore, these exosomes were efficiently endocytosed by tumor cells. Exosomes from Venofer-labeled MSCs expressing the yCDColon, two colonsUPRT gene in the presence of the prodrug 5-fluorocytosine inhibited tumor growth in a dose-dependent fashion. The treated tumor cells were also effectively ablated following induction of hyperthermia using an external alternating magnetic field. Cumulatively, we found that magnetic nanoparticles packaged into MSC exosomes are efficiently endocytosed by tumor cells, facilitating targeted tumor cell ablation via magnetically induced hyperthermia.

Alvarez, R., et al. (2015). "Characterization of the osteogenic potential of mesenchymal stem cells from human periodontal ligament based on cell surface markers." Int J Oral Sci **7**(4): 213-219.

Mesenchymal stem cell (MSC)-mediated therapy has been shown to be clinically effective in regenerating tissue defects. For improved regenerative therapy, it is critical to isolate homogenous populations of MSCs with high capacity to differentiate into appropriate tissues. The utilization of stem cell surface antigens provides a means to identify MSCs from various tissues. However, few surface markers that consistently isolate highly regenerative MSCs have been validated, making it challenging for routine clinical applications and making it all the more imperative to identify reliable surface markers. In this study, we used three surface marker combinations: CD51/CD140alpha, CD271, and STRO-1/CD146 for the isolation of homogenous populations of dental mesenchymal stem cells (DMSCs) from heterogeneous periodontal ligament cells (PDLCs). Fluorescence-activated cell sorting analysis revealed that 24% of PDLCs were CD51(+)/CD140alpha(+), 0.8% were CD271(+), and 2.4% were STRO-1(+)/CD146(+). Sorted cell populations were further assessed for their multipotent properties by inducing osteogenic and chondrogenic differentiation. All three subsets of isolated DMSCs exhibited differentiation capacity into osteogenic and chondrogenic lineages but with varying degrees. CD271(+) DMSCs demonstrated the greatest osteogenic potential with strong induction of osteogenic markers such as DLX5, RUNX2, and BGLAP. Our study provides evidence that surface marker combinations used in this study are sufficient markers for the isolation of DMSCs from PDLCs. These results provide important insight into using specific surface markers for identifying homogenous populations of DMSCs for their improved utilization in regenerative medicine.

Ame-Thomas, P., et al. (2007). "Human mesenchymal stem cells isolated from bone marrow and lymphoid organs support tumor B-cell growth: role of stromal cells in follicular lymphoma pathogenesis." Blood **109**(2): 693-702.

Accumulating evidence indicates that the cellular microenvironment plays a key role in follicular lymphoma (FL) pathogenesis, both within tumor lymph nodes (LNs) and in infiltrated bone marrow where ectopic LN-like reticular cells are integrated within

malignant B-cell nodular aggregates. In normal secondary lymphoid organs, specific stromal cell subsets provide a highly specialized microenvironment that supports immune response. In particular, fibroblastic reticular cells (FRCs) mediate immune cell migration, adhesion, and reciprocal interactions. The role of FRCs and their postulated progenitors, that is, bone marrow mesenchymal stem cells (MSCs), in FL remains unexplored. In this study, we investigated the relationships between FRCs and MSCs and their capacity to sustain malignant B-cell growth. Our findings strongly suggest that secondary lymphoid organs contain MSCs able to give rise to adipocytes, chondrocytes, osteoblasts, as well as fully functional B-cell supportive FRCs. In vitro, bone marrow-derived MSCs acquire a complete FRC phenotype in response to a combination of tumor necrosis factor-alpha and lymphotoxin-alpha1beta2. Moreover, MSCs recruit primary FL cells that, in turn, trigger their differentiation into FRCs, making them able to support malignant B-cell survival. Altogether, these new insights into the cross talk between lymphoma cells and their microenvironment could offer original therapeutic strategies.

Anderson, D. G., et al. (2013). "Human umbilical cord blood-derived mesenchymal stem cells in the cultured rabbit intervertebral disc: a novel cell source for disc repair." Am J Phys Med Rehabil **92**(5): 420-429.

OBJECTIVE: Back pain associated with symptomatic disc degeneration is a common clinical condition. Intervertebral disc (IVD) cell apoptosis and senescence increase with aging and degeneration. Repopulating the IVD with cells that could produce and maintain extracellular matrix would be an alternative therapy to surgery. The objective of this study was to determine the potential of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) as a novel cell source for disc repair. In this study, we intended to confirm the potential for hUCB-MSCs to differentiate and display a chondrocyte-like phenotype after culturing in micromass and after injection into the rabbit IVD explant culture. We also wanted to confirm hUCB-MSC survival after transplantation into the IVD explant culture. DESIGN: This study consisted of micromass cultures and in vitro rabbit IVD explant cultures to assess hUCB-MSC survival and differentiation to display chondrocyte-like phenotype. First, hUCB-MSCs were cultured in micromass and stained with Alcian blue dye. Second, to confirm cell survival, hUCB-MSCs were labeled with an infrared dye and a fluorescent dye before injection into whole rabbit IVD explants (host). IVD explants were then cultured for 4 wks. Cell survival was confirmed by two independent techniques: an imaging system detecting the infrared dye at the organ level and fluorescence microscopy detecting fluorescent dye at the cellular level. Cell viability was assessed by staining the explant with CellTracker green, a membrane-permeant tracer specific for live cells. Human type II collagen gene expression (from the graft) was assessed by polymerase chain reaction. RESULTS: We have shown that hUCB-MSCs cultured in micromass are stained blue with Alcian blue dye, which suggests that proteoglycan-rich extracellular matrix is produced. In the cultured rabbit IVD explants, hUCB-MSCs survived for at least 4 wks and expressed the human type II collagen gene, suggesting that the injected hUCB-MSCs are differentiating into a chondrocyte-like lineage. CONCLUSIONS: This study demonstrates the abiity of hUBC-MSCs to survive and assume a chondrocyte-like phenotype when injected into the rabbit IVD. These data support the potential for hUBC-MSCs as a cell source for disc repair. Further measures of the host response to the injection and studies in animal models are needed before trials in humans.

Anderson, S. B., et al. (2011). "The performance of human mesenchymal stem cells encapsulated in cell-degradable polymer-peptide hydrogels." Biomaterials **32**(14): 3564-3574.

Thiol-ene photopolymerization offers a unique platform for the formation of peptide-functionalized poly(ethylene glycol) hydrogels and the encapsulation, culture and differentiation of cells. Specifically, this photoinitiated polymerization scheme occurs at neutral pH and can be controlled both spatially and temporally. Here, we have encapsulated human mesenchymal stem cells (hMSCs) in matrix metalloproteinase (MMP) degradable and cell-adhesive hydrogels using thiol-ene photopolymerization. We find that hMSCs survive equally well in this system, regardless of MMP-degradability. When hMSCs are encapsulated in these cell-degradable hydrogels, they survive and are able to proliferate. In classic hMSC differentiation medias, hMSCs locally remodel their microenvironment and take on characteristic morphologies; hMSCs cultured in growth or osteogenic differentiation media are less round, as measured by elliptical form factor, and are smaller than hMSCs cultured in chondrogenic or adipogenic differentiation media. In addition, hMSCs encapsulated in completely cell-degradable hydrogels and cultured in osteogenic, chondrogenic, or adipogenic differentiation media generally express increased levels of specific differentiation markers as compared to cells in hydrogels that are not cell-degradable. These studies demonstrate the ability to culture and differentiate hMSCs in MMP-degradable hydrogels polymerized via a thiol-ene reaction scheme

and that increased cell-mediated hydrogel degradability facilitates directed differentiation of hMSCs.

Ardeshirylajimi, A., et al. (2017). "Renal Differentiation of Mesenchymal Stem Cells Seeded on Nanofibrous Scaffolds Improved by Human Renal Tubular Cell Lines-Conditioned Medium." ASAIO J **63**(3): 356-363.

Kidney injuries and renal dysfunctions are one of the most important clinical problems, and tissue engineering could be a valuable method for solving it. The objective of this study was to investigate the synergistic effect of renal cell line-conditioned medium and Polycaprolactone (PCL) nanofibers on renal differentiation of human mesenchymal stem cells (MSCs). In the current study, after stem cells isolation and characterization, PCL nanofibrous scaffold was fabricated using electrospinning methods and characterized morphologically, mechanically, and for biocompatibility. The renal differentiation of seeded MSCs on the surface of PCL nanofibers with and without human renal tubular cell lines-conditioned medium was investigated by evaluation of eight important renal-related genes expression by real-time reverse transcription polymerase chain reaction (RT-PCR) and immunocytochemistry. Fabricated nanofibrous scaffolds were good in all characterized items. Almost highest expression of all genes was detected in stem cells seeded on PCL under conditioned media in comparison with the stem cells seeded on PCL, tissue culture polystyrene (TCPS) under renal induction medium, and TCPS under conditioned medium. According to the results, PCL nanofibers in contribution with conditioned medium can provide the optimal conditions for renal differentiation of MSCs and could be a promising candidate for renal tissue engineering application.

Arpornmaeklong, P., et al. (2009). "Phenotypic characterization, osteoblastic differentiation, and bone regeneration capacity of human embryonic stem cell-derived mesenchymal stem cells." Stem Cells Dev **18**(7): 955-968.

To enhance the understanding of differentiation patterns and bone formation capacity of hESCs, we determined (1) the temporal pattern of osteoblastic differentiation of human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs), (2) the influence of a three-dimensional matrix on the osteogenic differentiation of hESC-MSCs in long-term culture, and (3) the bone-forming capacity of osteoblast-like cells derived from hESC-MSCs in calvarial defects. Incubation of hESC-MSCs in osteogenic medium induced osteoblastic differentiation of hESC-MSCs into mature osteoblasts in a similar chronological pattern to human bone marrow stromal cells and primary osteoblasts. Osteogenic differentiation was enhanced by culturing the cells on three-dimensional collagen scaffolds. Fluorescent-activated cell sorting of alkaline phosphatase expressing cells was used to obtain an enriched osteogenic cell population for in vivo transplantation. The identification of green fluorescence protein and expression of human-specific nuclear antigen in osteocytes in newly formed bone verified the role of transplanted human cells in the bone regeneration process. The current cell culture model and osteogenic cell enrichment method could provide large numbers of osteoprogenitor cells for analysis of differentiation patterns and cell transplantation to regenerate skeletal defects.

Arpornmaeklong, P., et al. (2017). "Effects of Titanium Surface Microtopography and Simvastatin on Growth and Osteogenic Differentiation of Human Mesenchymal Stem Cells in Estrogen-Deprived Cell Culture." Int J Oral Maxillofac Implants **32**(1): e35-e46.

PURPOSE: This study aimed to investigate the effects of titanium surface topography and simvastatin on growth and osteogenic differentiation of human bone marrow stromal cells (hBMSCs) in estrogen-deprived (ED) cell culture. MATERIALS AND METHODS: Human BMSCs were seeded on cell culture plates, smooth-surface titanium (Ti) disks, and sandblasted with large grits and acid etched (SLA)-surface Ti disks; and subsequently cultured in regular (fetal bovine serum [FBS]), ED, and ED-with 100 nM simvastatin (ED-SIM) culture media for 14 to 21 days. Live/dead cell staining, scanning electron microscope examination, and cell viability assay were performed to determine cell attachment, morphology, and growth. Expression levels of osteoblast-associated genes, Runx2 and bone sialoprotein and levels of alkaline phosphatase (ALP) activity, calcium content, and osteocalcin in culture media were measured to determine osteoblastic differentiation. Expression levels of bone morphogenetic protein-2 (BMP-2) were investigated to examine stimulating effects of simvastatin (n = 4 to 5, mean +/- SD). In vitro mineralization was verified by calcein staining. RESULTS: Human BMSCs exhibited different attachment and shapes on smooth and SLA titanium surfaces. Estrogen-deprived cell culture decreased cell attachment and growth, particularly on the SLA titanium surface, but cells were able to grow to reach confluence on day 21 in the ED-osteogenic (OS) culture medium. Promoting effects of the SLA titanium surface in ED-OS were significantly decreased. Simvastatin significantly increased osteogenic differentiation of human BMSCs on the SLA titanium surface in the ED-OS medium, and the promoting effects of simvastatin corresponded with the increasing

of BMP-2 gene expression on the SLA titanium surface in ED-OS-SIM culture medium. CONCLUSION: The ED cell culture model provided a well-defined platform for investigating the effects of hormones and growth factors on cells and titanium surface interaction. Titanium, the SLA surface, and simvastatin synergistically promoted osteoblastic differentiation of hBMSCs in ED condition and might be useful to promote osteointegration in osteoporotic bone.

Arufe, M. C., et al. (2010). "Chondrogenic potential of subpopulations of cells expressing mesenchymal stem cell markers derived from human synovial membranes." J Cell Biochem **111**(4): 834-845.

In this study we analyzed the chondrogenic potential of subpopulations of mesenchymal stem cells (MSCs) derived from human synovial membranes enriched for CD73, CD106, and CD271 markers. Subpopulations of human synovial membrane MSCs enriched for CD73, CD106, and CD271 markers were isolated using a cytometry sorter and characterized by flow cytometry for MSC markers. The expression of Sox9, Nanog, and Runx2 genes by these cells was measured by reverse transcriptase-polymerase chain reaction. The chondrogenesis of each subpopulation was assessed by culturing the cells in a defined medium to produce spontaneous spheroid formation and differentiation towards chondrocyte-like cells. The examination of the spheroids by histological and immunohistochemical analyses for collagen type II (COL2), aggrecan, collagen type I (COL1), metalloprotease 13 (MMP13), and collagen type X (COLX) levels were performed to assess their chondrogenesis capacity. The adipogenesis and osteogenesis potential of each subpopulation was determined using commercial media; the resulting cells were stained with oil red O or red alizarin to test the degree of differentiation. The subpopulations had different profiles of cells positive for the MSC markers CD44, CD69, CD73, CD90, and CD105 and showed different expression levels of the genes Sox9, Nanog, and Runx2 involved in chondrogenesis, undifferentiation, and osteoblastogenesis, respectively. Immunohistochemical analysis demonstrated that COL1, COL2, COLX, MMP13, and aggrecan were expressed in the spheroids as soon as 14 days of culture. The CD271(+) subpopulation expressed the highest levels of COL2 staining compared to the other subpopulations. CD105 and Runx2 were shown by immunohistochemistry and genetic analysis to have significantly higher expression CD271(+) subpopulation than the other subpopulations. Spheroids formed from CD271-enriched and CD73-enriched MSCs from normal human synovial membranes mimic the native cartilage extracellular matrix more closely than CD106(+) MSCs and are possible candidates for use in cartilage tissue engineering. Both cell types have potential for promoting the differentiation of MSCs into chondrocytes, presenting new possibilities for achieving intrinsic cartilage repair.

Askar, S. F., et al. (2013). "Engraftment patterns of human adult mesenchymal stem cells expose electrotonic and paracrine proarrhythmic mechanisms in myocardial cell cultures." Circ Arrhythm Electrophysiol **6**(2): 380-391.

BACKGROUND: After intramyocardial injection, mesenchymal stem cells (MSCs) may engraft and influence host myocardium. However, engraftment rate and pattern of distribution are difficult to control in vivo, hampering assessment of potential adverse effects. In this study, the role of the engraftment patterns of MSCs on arrhythmicity in controllable in vitro models is investigated. METHODS AND RESULTS: Cocultures of 4x10(5) neonatal rat cardiomyocytes and 7% or 28% adult human MSCs (hMSCs) in diffuse or clustered distribution patterns were prepared. Electrophysiological effects were studied by optical mapping and patch-clamping. In diffuse cocultures, hMSCs dose-dependently decreased neonatal rat cardiomyocyte excitability, slowed conduction, and prolonged action potential duration until 90% repolarization (APD90). Triggered activity (14% versus 0% in controls) and increased inducibility of re-entry (53% versus 6% in controls) were observed in 28% hMSC cocultures. MSC clusters increased APD90, slowed conduction locally, and increased re-entry inducibility (23%), without increasing triggered activity. Pharmacological heterocellular electric uncoupling increased excitability and conduction velocity to 133% in 28% hMSC cocultures, but did not alter APD90. Transwell experiments showed that hMSCs dose-dependently increased APD90, APD dispersion, inducibility of re-entry and affected specific ion channel protein levels, whereas excitability was unaltered. Incubation with hMSC-derived exosomes did not increase APD in neonatal rat cardiomyocyte cultures. CONCLUSIONS: Adult hMSCs affect arrhythmicity of neonatal rat cardiomyocyte cultures by heterocellular coupling leading to depolarization-induced conduction slowing and by direct release of paracrine factors that negatively affect repolarization rate. The extent of these detrimental effects depends on the number and distribution pattern of hMSCs. These results suggest that caution should be urged against potential adverse effects of myocardial hMSC engraftment.

Bajetto, A., et al. (2017). "Different Effects of Human Umbilical Cord Mesenchymal Stem Cells on Glioblastoma Stem Cells by Direct Cell Interaction or

Via Released Soluble Factors." Front Cell Neurosci **11**: 312.

Glioblastoma (GBM), the most common primary brain tumor in adults, is an aggressive, fast-growing and highly vascularized tumor, characterized by extensive invasiveness and local recurrence. In GBM and other malignancies, cancer stem cells (CSCs) are believed to drive invasive tumor growth and recurrence, being responsible for radio- and chemo-therapy resistance. Mesenchymal stem cells (MSCs) are multipotent progenitors that exhibit tropism for tumor microenvironment mediated by cytokines, chemokines and growth factors. Initial studies proposed that MSCs might exert inhibitory effects on tumor development, although, to date, contrasting evidence has been provided. Different studies reported either MSC anti-tumor activity or their support to tumor growth. Here, we examined the effects of umbilical cord (UC)-MSCs on in vitro GBM-derived CSC growth, by direct cell-to-cell interaction or indirect modulation, via the release of soluble factors. We demonstrate that UC-MSCs and CSCs exhibit reciprocal tropism when co-cultured as 3D spheroids and their direct cell interaction reduces the proliferation of both cell types. Contrasting effects were obtained by UC-MSC released factors: CSCs, cultured in the presence of conditioned medium (CM) collected from UC-MSCs, increased proliferation rate through transient ERK1/2 and Akt phosphorylation/activation. Analysis of the profile of the cytokines released by UC-MSCs in the CM revealed a strong production of molecules involved in inflammation, angiogenesis, cell migration and proliferation, such as IL-8, GRO, ENA-78 and IL-6. Since CXC chemokine receptor 2 (CXCR2), a receptor shared by several of these ligands, is expressed in GBM CSCs, we evaluated its involvement in CSC proliferation induced by UC-MSC-CM. Using the CXCR2 antagonist SB225002, we observed a partial but statistically significant inhibition of CSC proliferation and migration induced by the UC-MSC-released cytokines. Conversely, CXCR2 blockade did not reduce the reciprocal tropism between CSCs and UC-MSCs grown as spheroids. In conclusion, we show that direct (cell-to-cell contact) or indirect (via the release of soluble factors) interactions between GBM CSCs and UC-MSCs in co-culture produce divergent effects on cell growth, invasion and migration, with the former mainly causing an inhibitory response and the latter a stimulatory one, involving a paracrine activation of CXCR2.

Bajpai, V. K., et al. (2012). "Functional vascular smooth muscle cells derived from human induced pluripotent stem cells via mesenchymal stem cell intermediates." Cardiovasc Res **96**(3): 391-400.

AIMS: Smooth muscle cells (SMC) play an important role in vascular homeostasis and disease. Although adult mesenchymal stem cells (MSC) have been used as a source of contractile SMC, they suffer from limited proliferation potential and culture senescence, particularly when originating from older donors. By comparison, human induced pluripotent stem cells (hiPSC) can provide an unlimited source of functional SMC for autologous cell-based therapies and for creating models of vascular disease. Our goal was to develop an efficient strategy to derive functional, contractile SMC from hiPSC. METHODS AND RESULTS: We developed a robust, stage-wise, feeder-free strategy for hiPSC differentiation into functional SMC through an intermediate stage of multipotent MSC, which could be coaxed to differentiate into fat, bone, cartilage, and muscle. At this stage, the cells were highly proliferative and displayed higher clonogenic potential and reduced senescence when compared with parental hair follicle mesenchymal stem cells. In addition, when exposed to differentiation medium, the myogenic proteins such as alpha-smooth muscle actin, calponin, and myosin heavy chain were significantly upregulated and displayed robust fibrillar organization, suggesting the development of a contractile phenotype. Indeed, tissue constructs prepared from these cells exhibited high levels of contractility in response to receptor- and non-receptor-mediated agonists. CONCLUSION: We developed an efficient stage-wise strategy that enabled hiPSC differentiation into contractile SMC through an intermediate population of clonogenic and multipotent MSC. The high yield of MSC and SMC derivation suggests that our strategy may facilitate an acquisition of the large numbers of cells required for regenerative medicine or for studying vascular disease pathophysiology.

Bak, X. Y., et al. (2011). "Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for prodrug gene therapy of glioblastoma." Hum Gene Ther **22**(11): 1365-1377.

Mesenchymal stem cells (MSCs) possess tumor-tropic properties and consequently have been used to deliver therapeutic agents for cancer treatment. Their potential in cancer therapy highlights the need for a consistent and renewable source for the production of uniform human MSCs suitable for clinical applications. In this study, we seek to investigate whether human embryonic stem cells can be used as a cell source to fulfill this goal. We generated MSC-like cells from two human embryonic stem cell lines, HuES9 and H1, and observed that MSC-like cells derived from human embryonic stem cells were able to migrate into human glioma intracranial xenografts after being injected into the cerebral hemisphere contralateral to the tumor

inoculation site. We engineered these cells with baculoviral and lentiviral vectors, respectively, for transient and stable expression of the herpes simplex virus thymidine kinase gene. In tumor-bearing mice the engineered MSC-like cells were capable of inhibiting tumor growth and prolonging survival in the presence of ganciclovir after they were injected either directly into the xenografts or into the opposite hemisphere. Our findings suggest that human embryonic stem cell-derived MSCs may be a viable and attractive alternative for large-scale derivation of targeting vehicles for cancer therapy.

Balci, D. and A. Can (2013). "The assessment of cryopreservation conditions for human umbilical cord stroma-derived mesenchymal stem cells towards a potential use for stem cell banking." Curr Stem Cell Res Ther **8**(1): 60-72.

Human umbilical cord stroma-derived mesenchymal stem cells (hUCS-MSCs) are considered as a remarkable and promising stem cell source to be potentially used in cellular therapies. While no graft rejection has been reported in the recipient organism even in xeno-transplantation studies, attenuate tumor cell growth and gene transfers have been experimentally shown. In this study, we have demonstrated a reliable, reproducible and efficient cryopreservation method of hUCS-MSCs resulting in one of the highest cell survival rates reported so far. Conventional, computer-controlled multistep slow freezing (MSSF), and vitrification methods were comparatively tested using cell permeable [dimethylsulfoxide (DMSO), ethylene glycol] and impermeable [trehalose, sucrose, hydroxyethyl starch (HES), human serum albumin] cryoprotectant agents (CPAs). After determining the ice nucleation point for each solution, latent heat evolution was suppressed during freezing, followed by a cooling process to -40 degrees C at 1 degrees C/min or 0.3 degrees C/min. The efficiency of the cryopreservation techniques used was determined by cell viability and proliferation assays, the expression of cell surface markers, cytoskeletal proteins and chromosome alignments. The cell survival rate was found to be highest (87 +/- 5%) by MSSF with sucrose (0.1 M) +DMSO (10%) at 1 degrees C/min freezing rate. In this group, no significant difference was noted before and after the cryopreservation in cell morphology, cytokeratin, vimentin, and alpha-smooth muscle actin profiles and the expressions of CD105, CD90, CD73, CD29 and HLA-DR. Second highest cell survival ratio (85 +/- 6%) was obtained in DMSO (10%) alone at 1 degrees C/min freezing rate. Interestingly, poor (18 +/- 15%) cell survival rates were obtained after vitrification. Cumulatively, results indicated that MSSF favors the other freezing protocols with an addition of sucrose or DMSO alone depending on the freezing rate used.

Barbet, R., et al. (2012). "Expression of the 49 human ATP binding cassette (ABC) genes in pluripotent embryonic stem cells and in early- and late-stage multipotent mesenchymal stem cells: possible role of ABC plasma membrane transporters in maintaining human stem cell pluripotency." Cell Cycle **11**(8): 1611-1620.

The 49-member human ATP binding cassette (ABC) gene family encodes 44 membrane transporters for lipids, ions, peptides or xenobiotics, four translation factors without transport activity, as they lack transmembrane domains, and one pseudogene. To understand the roles of ABC genes in pluripotency and multipotency, we performed a sensitive qRT-PCR analysis of their expression in embryonic stem cells (hESCs), bone marrow-derived mesenchymal stem cells (hMSCs) and hESC-derived hMSCs (hES-MSCs). We confirm that hES-MSCs represent an intermediate developmental stage between hESCs and hMSCs. We observed that 44 ABCs were significantly expressed in hESCs, 37 in hES-MSCs and 35 in hMSCs. These variations are mainly due to plasma membrane transporters with low but significant gene expression: 18 are expressed in hESCs compared with 16 in hES-MSCs and 8 in hMSCs, suggesting important roles in pluripotency. Several of these ABCs shared similar substrates but differ regarding gene regulation. ABCA13 and ABCB4, similarly to ABCB1, could be new markers to select primitive hMSCs with specific plasma membrane transporter (low) phenotypes. ABC proteins performing basal intracellular functions, including translation factors and mitochondrial heme transporters, showed the highest constant gene expression among the three populations. Peptide transporters in the endoplasmic reticulum, Golgi and lysosome were well expressed in hESCs and slightly upregulated in hMSCs, which play important roles during the development of stem cell niches in bone marrow or meningeal tissue. These results will be useful to study specific cell cycle regulation of pluripotent stem cells or ABC dysregulation in complex pathologies, such as cancers or neurological disorders.

Basu, J., et al. (2011). "Expansion of the human adipose-derived stromal vascular cell fraction yields a population of smooth muscle-like cells with markedly distinct phenotypic and functional properties relative to mesenchymal stem cells." Tissue Eng Part C Methods **17**(8): 843-860.

Adipose tissue contains a heterogeneous cell population composed of endothelial cells, adipocytes, smooth muscle cells (SMC), and mesenchymal

progenitors and stromal cells that meet the criteria put forth by the International Society for Cellular Therapy as defining mesenchymal stem cells (MSC). In this study, we expanded the stromal vascular fraction (SVF) of human adipose tissue and characterized the resulting adherent primary cell cultures by quantitative reverse transcription-polymerase chain reaction, antigen expression, protein fingerprinting, growth kinetics, in vitro tri-lineage differentiation bioactivity, and functional responses to small molecules modulating SMC-related developmental pathways and compared the results to those obtained with functionally validated MSC cultures. SVF-derived initial cultures (P0) were expanded in a defined medium that was not optimized for MSC growth conditions, neither were recombinant cytokines or growth factors added to the media to direct differentiation. The adherent cell cultures derived from SVF expansion under these conditions had markedly distinct phenotypic and biological properties relative to functionally validated MSC cultures. SVF-derived adherent cell cultures retained characteristics consistent with the SMC subpopulation within adipose tissue--phenotype, gene, and protein expression--that were independent of passage number and source of SVF (n=4 independent donors). SVF-derived cells presented significantly less robust in vitro tri-lineage differentiation bioactivity relative to validated MSC. Expanded SVF cells and MSC had opposite responses to the thromboxane A2 mimetic U46619, demonstrating an unambiguous functional distinction between the two cell types. Taken together, these data support the conclusions that SVF cells expanded under the conditions described in these studies are accurately described as adipose-derived SMC and represent a cellular subpopulation of adipose SVF that is separate and distinct from other classes of adipose-derived cells.

Batten, P., et al. (2006). "Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: relevance to tissue engineering human heart valves." Tissue Eng **12**(8): 2263-2273.

To generate an "off the shelf" tissue-engineered heart valve, the cells would need to be of allogeneic origin. Here, we report the possibility of using human bone marrow-derived mesenchymal stem cells (MSCs) as a suitable allogeneic cell source for tissue-engineered heart valves. Proliferative responses of primary and primed CD4+ T cells to allogeneic MSCs were examined. A protein microarray system was used to detect soluble factors from supernatants collected from the T cell assays. MSCs are poor stimulators of primary and primed CD4+ T cell proliferation, despite provision of B7-1 trans-co-stimulation. MSCs not only directly inhibited primary and primed T cell responses to allogeneic peripheral blood mononuclear cells (PBMCs), but 24-h pre-culture of T cells with MSCs suppressed subsequent T cell proliferative responses to allogeneic PBMCs in a contact-dependent manner. Analysis of supernatants revealed a distinctly different cytokine profile after co-culture of T cells with MSCs than with PBMCs or endothelial cells. Pro-inflammatory Th1 cytokines interleukin (IL)-1alpha and beta, interferon (IFN)gamma, and tumor necrosis factor (TNF)alpha were downregulated, whereas, anti-inflammatory Th2 cytokines IL-3, IL-5, IL-10, and IL-13 and the Th2 chemokine I-309, a chemoattractant for regulatory T cells, were upregulated. Further analysis revealed that after co-culture with MSCs, the T cells exhibited a regulatory phenotype (CD4+ CD25(lo) CD69(lo) FoxP3+). MSCs downregulate T cell responses through direct contact and secretion of anti-inflammatory and tolerogenic cytokines, which may involve the recruitment of regulatory T cells. This implies that allogeneic MSCs could be a suitable cell source for tissue engineering a heart valve.

Bensidhoum, M., et al. (2004). "Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment." Blood **103**(9): 3313-3319.

The Stro-1 antigen potentially defines a mesenchymal stem cell (MSC) progenitor subset. We here report on the role of human ex vivo-expanded selected Stro-1(+) or Stro-1(-) MSC subsets on the engraftment of human CD34(+) cord blood cells in the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mouse model. The data show that cotransplantation of expanded Stro-1(-) cells with CD34(+) cells resulted in a significant increase of human CD45, CD34, CD19, and CD11b cells detected in blood or in bone marrow (BM) and spleen as compared with the infusion of CD34(+) cells alone. Infusion into mice of expanded Stro-1(+) and Stro-1(-) cells (without CD34(+) cells) showed that the numbers of Stro-1(+)-derived (as assessed by DNA analysis of human beta-globin with quantitative polymerase chain reaction [PCR]) were higher than Stro-1(-)-derived cells in spleen, muscles, BM, and kidneys, while more Stro-1(-)-derived than Stro-1(+)-derived cells were found in lungs. The transduction of expanded Stro-1(+) cells with an enhanced green fluorescent protein (eGFP) gene did not modify their cytokine release and their homing in NOD/SCID mouse tissues. The difference between the hematopoietic support and the homing capabilities of expanded Stro-1(+) and Stro-1(-) cells may be of importance for clinical therapeutic applications: Stro-1(+) cells may rather be used for

gene delivery in tissues while Stro-1(-) cells may rather be used to support hematopoietic engraftment.

Benvenuto, F., et al. (2015). "Human mesenchymal stem cells target adhesion molecules and receptors involved in T cell extravasation." Stem Cell Res Ther **6**: 245.

INTRODUCTION: Systemic delivery of bone marrow-derived mesenchymal stem cells (MSC) seems to be of benefit in the treatment of multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS) sustained by migration of T cells across the brain blood barrier (BBB) and subsequent induction of inflammatory lesions into CNS. MSC have been found to modulate several effector functions of T cells. In this study, we investigated the effects of MSC on adhesion molecules and receptors on T cell surface that sustain their transendothelial migration. METHODS: We used different co-culture methods combined with real-time PCR and flow cytometry to evaluate the expression both at the mRNA and at the plasma-membrane level of alpha4 integrin, beta2 integrin, ICAM-1 and CXCR3. In parallel, we assessed if MSC are able to modulate expression of adhesion molecules on the endothelial cells that interact with T cells during their transendothelial migration. RESULTS: Our in vitro analyses revealed that MSC: (i) inhibit proliferation and activation of both peripheral blood mononuclear cells (PBMC) and CD3(+)-selected lymphocytes through the release of soluble factors; (ii) exert suppressive effects on those surface molecules highly expressed by activated lymphocytes and involved in transendothelial migration; (iii) inhibit CXCL10-driven chemotaxis of CD3(+) cells; (iv) down-regulated expression of adhesion molecules on endothelial cells. CONCLUSIONS: Taken together, these data demonstrate that the immunosuppressive effect of MSC does not exclusively depends on their anti-proliferative activity on T cells, but also on the impairment of leukocyte migratory potential through the inhibition of the adhesion molecules and receptors that are responsible for T cell trafficking across BBB. This could suggest a new mechanism through which MSC modulate T cell responses.

Bergante, S., et al. (2014). "Gangliosides as a potential new class of stem cell markers: the case of GD1a in human bone marrow mesenchymal stem cells." J Lipid Res **55**(3): 549-560.

Owing to their exposure on the cell surface and the possibility of being directly recognized with specific antibodies, glycosphingolipids have aroused great interest in the field of stem cell biology. In the search for specific markers of the differentiation of human bone marrow mesenchymal stem cells (hBMSCs) toward osteoblasts, we studied their glycosphingolipid pattern, with particular attention to gangliosides. After lipid extraction and fractionation, gangliosides, metabolically (3)H-labeled in the sphingosine moiety, were separated by high-performance TLC and chemically characterized by MALDI MS. Upon induction of osteogenic differentiation, a 3-fold increase of ganglioside GD1a was observed. Therefore, the hypothesis of GD1a involvement in hBMSCs commitment toward the osteogenic phenotype was tested by comparison of the osteogenic propensity of GD1a-highly expressing versus GD1a-low expressing hBMSCs and direct addition of GD1a in the differentiation medium. It was found that either the high expression of GD1a in hBMSCs or the addition of GD1a in the differentiation medium favored osteogenesis, providing a remarkable increase of alkaline phosphatase. It was also observed that ganglioside GD2, although detectable in hBMSCs by immunohistochemistry with an anti-GD2 antibody, could not be recognized by chemical analysis, likely reflecting a case, not uncommon, of molecular mimicry.

Bertoncini, P., et al. (2012). "Early adhesion of human mesenchymal stem cells on TiO(2) surfaces studied by single-cell force spectroscopy measurements." J Mol Recognit **25**(5): 262-269.

Understanding the interactions involved in the adhesion of living cells on surfaces is essential in the field of tissue engineering and biomaterials. In this study, we investigate the early adhesion of living human mesenchymal stem cells (hMSCs) on flat titanium dioxide (TiO(2)) and on nanoporous crystallized TiO(2) surfaces with the use of atomic force microscopy-based single-cell force spectroscopy measurements. The choice of the substrate surfaces was motivated by the fact that implants widely used in orthopaedic and dental surgery are made in Ti and its alloys. Nanoporous TiO(2) surfaces were produced by anodization of Ti surfaces. In a typical force spectroscopy experiment, one living hMSC, immobilized onto a fibronectine-functionalized tipless lever is brought in contact with the surface of interest for 30 s before being detached while recording force-distance curves. Adhesion of hMSCs on nanoporous TiO(2) substrates having inner pore diameter of 45 nm was lower by approximately 25% than on TiO(2) flat surfaces. Force-distance curves exhibited also force steps that can be related to the pulling of membrane tethers from the cell membrane. The mean force step was equal to 35 pN for a given speed independently of the substrate surface probed. The number of tethers observed was substrate dependent. Our results suggest that the strength of the initial adhesion between hMSCs and flat or nanoporous TiO(2) surfaces is driven by the adsorption of proteins deposited from serum in the culture media.

Beyth, S., et al. (2005). "Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness." Blood **105**(5): 2214-2219.

Infusion of either embryonic or mesenchymal stem cells prolongs the survival of organ transplants derived from stem cell donors and prevents graft-versus-host-disease (GVHD). An in-depth mechanistic understanding of this tolerization phenomenon could lead to novel cell-based therapies for transplantation. Here we demonstrate that while human mesenchymal stem cells (hMSCs) can promote superantigen-induced activation of purified T cells, addition of antigen-presenting cells (APCs; either monocytes or dendritic cells) to the cultures inhibits the T-cell responses. This contact- and dose-dependent inhibition is accompanied by secretion of large quantities of interleukin (IL)-10 and aberrant APC maturation, which can be partially overridden by the addition of factors that promote APC maturation (ie, lipopolysaccharide [LPS] or anti-CD40 monoclonal antibody [mAb]). Thus, our data support an immunoregulatory mechanism wherein hMSCs inhibit T cells indirectly by contact-dependent induction of regulatory APCs with T-cell-suppressive properties. Our data may reveal a physiologic phenomenon whereby the development of a distinct APC population is regulated by the tissue's cellular microenvironment.

Binato, R., et al. (2013). "Stability of human mesenchymal stem cells during in vitro culture: considerations for cell therapy." Cell Prolif **46**(1): 10-22.

Ex vivo expansion and manipulation of human mesenchymal stem cells are important approaches to immunoregulatory and regenerative cell therapies. Although these cells show great potential for use, issues relating to their overall nature emerge as problems in the field. The need for extensive cell quantity amplification in vitro to obtain sufficient cell numbers for use, poses a risk of accumulating genetic and epigenetic abnormalities that could lead to sporadic malignant cell transformation. In this study, we have examined human mesenchymal stem cells derived from bone marrow, over extended culture time, using cytogenetic analyses, mixed lymphocyte reactions, proteomics and gene expression assays to determine whether the cultures would retain their potential for use in subsequent passages. Results indicate that in vitro cultures of these cells demonstrated chromosome variability after passage 4, but their immunomodulatory functions and differentiation capacity were maintained. At the molecular level, changes were observed from passage 5 on, indicating initiation of differentiation. Together, these results lead to the hypothesis that human mesenchymal stem cells cultures can be used successfully in cell therapy up to passage 4. However, use of cells from higher passages would have to be analysed case by case.

Bindslev, L., et al. (2006). "Labelling of human mesenchymal stem cells with indium-111 for SPECT imaging: effect on cell proliferation and differentiation." Eur J Nucl Med Mol Imaging **33**(10): 1171-1177.

PURPOSE: Stem cell therapy seems to be a new treatment option within cardiac diseases to improve myocardial perfusion and function. However, the delivery and traceability of the cells represent a problem. Radioactive labelling with 111In could be a method for tracking mesenchymal stem cells (MSCs). However, 111In could influence the viability and differentiation capacity of MSCs, which would limit its use. Therefore, the aim of this study was to evaluate the influence of 111In labelling in doses relevant for SPECT imaging in humans on the viability and differentiation capacity of human MSCs. METHODS AND RESULTS: Human MSCs isolated from bone marrow were incubated with 111In-tropolone (15-800 Bq/cell). The labelling efficiency was approximately 25% with 30 Bq/cell 111In. The MSC doubling time was 1.04+/-0.1 days and was not influenced by 111In within the range 15-260 Bq/cell. Using 30 Bq 111In/cell it was possible to label MSCs to a level relevant for clinical scintigraphic use. With this dose, 111In had no effect on characteristic surface and intracellular markers of cultured MSCs analysed both by flow cytometry and by real-time polymerase chain reaction. Further, the labelled MSCs differentiated towards endothelial cells and formed vascular structures. CONCLUSION: It is possible to label human MSCs with 111In for scintigraphic tracking of stem cells delivered to the heart in clinical trials without affecting the viability and differentiation capacity of the MSCs. This creates an important tool for the control of stem cell delivery and dose response in clinical cardiovascular trials.

Block, T. J., et al. (2017). "Restoring the quantity and quality of elderly human mesenchymal stem cells for autologous cell-based therapies." Stem Cell Res Ther **8**(1): 239.

BACKGROUND: Degenerative diseases are a major public health concern for the aging population and mesenchymal stem cells (MSCs) have great potential for treating many of these diseases. However, the quantity and quality of MSCs declines with aging, limiting the potential efficacy of autologous MSCs for treating the elderly population. METHODS: Human bone marrow (BM)-derived MSCs from young and elderly donors were obtained and characterized using

standard cell surface marker criteria (CD73, CD90, CD105) as recommended by the International Society for Cellular Therapy (ISCT). The elderly MSC population was isolated into four subpopulations based on size and stage-specific embryonic antigen-4 (SSEA-4) expression using fluorescence-activated cell sorting (FACS), and subpopulations were compared to the unfractionated young and elderly MSCs using assays that evaluate MSC proliferation, quality, morphology, intracellular reactive oxygen species, beta-galactosidase expression, and adenosine triphosphate (ATP) content. RESULTS: The ISCT-recommended cell surface markers failed to detect any differences between young and elderly MSCs. Here, we report that elderly MSCs were larger in size and displayed substantially higher concentrations of intracellular reactive oxygen species and beta-galactosidase expression and lower amounts of ATP and SSEA-4 expression. Based on these findings, cell size and SSEA-4 expression were used to separate the elderly MSCs into four subpopulations by FACS. The original populations (young and elderly MSCs), as well as the four subpopulations, were then characterized before and after culture on tissue culture plastic and BM-derived extracellular matrix (BM-ECM). The small SSEA-4-positive subpopulation representing ~ 8% of the original elderly MSC population exhibited a "youthful" phenotype that was similar to that of young MSCs. The biological activity of this elderly subpopulation was inhibited by senescence-associated factors produced by the unfractionated parent population. After these "youthful" cells were isolated and expanded (three passages) on a "young microenvironment" (i.e., BM-ECM produced by BM cells from young donors), the number of cells increased approximately 17,000-fold to 3 x 10(9) cells and retained their "youthful" phenotype. CONCLUSIONS: These results suggest that it is feasible to obtain large numbers of high-quality autologous MSCs from the elderly population and establish personal stem cell banks that will allow serial infusions of "rejuvenated" MSCs for treating age-related diseases.

Bochev, I., et al. (2008). "Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro." Cell Biol Int **32**(4): 384-393.

Mesenchymal stem cells (MSC) have been characterized as multipotent cells which are able to differentiate into several mesodermal and nonmesodermal lineage cells and this feature along with their extensive growth and comprehensive immunomodulatory properties establish them as a promising tool for therapeutic applications, including cell-based tissue engineering and treatment of immune-mediated disorders. Although bone marrow (BM) is the most common MSC source, cells with similar characteristics have been shown to be present in several other adult tissues. Adipose tissue (AT), large quantities of which can be easily obtained, represents an attractive alternative to BM in isolating adipose tissue-derived MSC (AT-MSC). BM-MSCs and AT-MSCs share some immunomodulatory properties as they are both not inherently immunogenic and suppress the proliferation of alloantigen- or mitogen-stimulated T-cells. Our purpose was to comparatively examine under appropriate in vitro conditions, phenotypes, morphology and some functional properties of BM-MSCs and AT-MSCs, such as differentiation potential and especially the ability to suppress the immunoglobulin production by mitogen-stimulated B-cells. While the morphological, immunophenotypical, colony-forming and adipogenic characteristics of both types of cells were almost identical, AT-MSCs showed less potential for osteogenic differentiation than BM-MSCs. We found that AT-MSCs not only inhibited the Ig-production but also suppressed this B-cell function to a much greater extent compared to BM-MSC. This finding supports the potential role of AT-MSCs as an alternative to BM-MSCs for clinical purposes.

Boxall, S. and E. Jones (2015). "The use of multiparameter flow cytometry and cell sorting to characterize native human bone marrow mesenchymal stem cells (MSC)." Methods Mol Biol **1235**: 121-130.

This chapter describes a method for identification, phenotypic analysis, and cell sorting of rare mesenchymal stem cells (MSCs) from human bone marrow (BM) aspirates. The native BM MSC population is identified based on the CD45(-/low)CD271(+) phenotype. The method consists of three related procedures: Procedure 1 involves a microbead-based pre-enrichment step. Two other procedures describe direct flow cytometric analysis of MSCs following the isolation of the mononuclear cell (MNC) fraction (Procedure 2) or more rapidly, following a simple ammonium chloride-based red cell lysis (Procedure 3). Recently described multi-lineage transcript expression in the CD45(-/low)CD271(+) cells suggests that the native BM MSC fraction could be further subdivided into functionally distinct subpopulations. The present protocols are hoped to help MSC biologists to enter this exciting field of research and to take it forward towards a better understanding of MSC biology in vivo.

Boyd, N. L., et al. (2011). "Microvascular mural cell functionality of human embryonic stem cell-derived mesenchymal cells." Tissue Eng Part A **17**(11-12): 1537-1548.

Microvascular mural or perivascular cells are required for the stabilization and maturation of the

remodeling vasculature. However, much less is known about their biology and function compared to large vessel smooth muscle cells. We have developed lines of multipotent mesenchymal cells from human embryonic stem cells (hES-MC); we hypothesize that these can function as perivascular mural cells. Here we show that the derived cells do not form teratomas in SCID mice and independently derived lines show similar patterns of gene expression by microarray analysis. When exposed to platelet-derived growth factor-BB, the platelet-derived growth factor receptor beta is activated and hES-MC migrate in response to a gradient. We also show that in a serum-free medium, transforming growth factor beta1 (TGFbeta1) induces robust expression of multiple contractile proteins (alpha smooth muscle actin, smooth muscle myosin heavy chain, smooth muscle 22alpha, and calponin). TGFbeta1 signaling is mediated through the TGFbetaR1/Alk5 pathway as demonstrated by inhibition of alpha smooth muscle actin expression by treatment of the Alk5-specific inhibitor SB525334 and stable retroviral expression of the Alk5 dominant negative (K232R). Coculture of human umbilical vein endothelial cell (HUVEC) with hES-MC maintains network integrity compared to HUVEC alone in three-dimensional collagen I-fibronectin by paracrine signaling. Using high-resolution laser confocal microscopy, we show that hES-MC also make direct contact with HUVEC. This demonstrates that hESC-derived mesenchymal cells possess the molecular machinery expected in a perivascular progenitor cells and can play a functional role in stabilizing EC networks in in vitro three-dimensional culture.

Bravo, B., et al. (2016). "Restrained Th17 response and myeloid cell infiltration into the central nervous system by human decidua-derived mesenchymal stem cells during experimental autoimmune encephalomyelitis." Stem Cell Res Ther **7**: 43.

BACKGROUND: Multiple sclerosis is a widespread inflammatory demyelinating disease. Several immunomodulatory therapies are available, including interferon-beta, glatiramer acetate, natalizumab, fingolimod, and mitoxantrone. Although useful to delay disease progression, they do not provide a definitive cure and are associated with some undesirable side-effects. Accordingly, the search for new therapeutic methods constitutes an active investigation field. The use of mesenchymal stem cells (MSCs) to modify the disease course is currently the subject of intense interest. Decidua-derived MSCs (DMSCs) are a cell population obtained from human placental extraembryonic membranes able to differentiate into the three germ layers. This study explores the therapeutic potential of DMSCs. METHODS: We used the experimental autoimmune encephalomyelitis (EAE) animal model to evaluate the effect of DMSCs on clinical signs of the disease and on the presence of inflammatory infiltrates in the central nervous system. We also compared the inflammatory profile of spleen T cells from DMSC-treated mice with that of EAE control animals, and the influence of DMSCs on the in vitro definition of the Th17 phenotype. Furthermore, we analyzed the effects on the presence of some critical cell types in central nervous system infiltrates. RESULTS: Preventive intraperitoneal injection of DMSCs resulted in a significant delay of external signs of EAE. In addition, treatment of animals already presenting with moderate symptoms resulted in mild EAE with reduced disease scores. Besides decreased inflammatory infiltration, diminished percentages of CD4(+)IL17(+), CD11b(+)Ly6G(+) and CD11b(+)Ly6C(+) cells were found in infiltrates of treated animals. Early immune response was mitigated, with spleen cells of DMSC-treated mice displaying low proliferative response to antigen, decreased production of interleukin (IL)-17, and increased production of the anti-inflammatory cytokines IL-4 and IL-10. Moreover, lower RORgammaT and higher GATA-3 expression levels were detected in DMSC-treated mice. DMSCs also showed a detrimental influence on the in vitro definition of the Th17 phenotype. CONCLUSIONS: DMSCs modulated the clinical course of EAE, modified the frequency and cell composition of the central nervous system infiltrates during the disease, and mediated an impairment of Th17 phenotype establishment in favor of the Th2 subtype. These results suggest that DMSCs might provide a new cell-based therapy for the control of multiple sclerosis.

Brenner, A. K., et al. (2016). "The Complexity of Targeting PI3K-Akt-mTOR Signalling in Human Acute Myeloid Leukaemia: The Importance of Leukemic Cell Heterogeneity, Neighbouring Mesenchymal Stem Cells and Immunocompetent Cells." Molecules **21**(11).

Therapeutic targeting of PI3K-Akt-mTOR is considered a possible strategy in human acute myeloid leukaemia (AML); the most important rationale being the proapoptotic and antiproliferative effects of direct PI3K/mTOR inhibition observed in experimental studies of human AML cells. However, AML is a heterogeneous disease and these effects caused by direct pathway inhibition in the leukemic cells are observed only for a subset of patients. Furthermore, the final effect of PI3K-Akt-mTOR inhibition is modulated by indirect effects, i.e., treatment effects on AML-supporting non-leukemic bone marrow cells. In this article we focus on the effects of this treatment on mesenchymal stem cells (MSCs) and monocytes/macrophages; both these cell types are parts

of the haematopoietic stem cell niches in the bone marrow. MSCs have unique membrane molecule and constitutive cytokine release profiles, and mediate their support through bidirectional crosstalk involving both cell-cell contact and the local cytokine network. It is not known how various forms of PI3K-Akt-mTOR targeting alter the molecular mechanisms of this crosstalk. The effect on monocytes/macrophages is also difficult to predict and depends on the targeted molecule. Thus, further development of PI3K-Akt-mTOR targeting into a clinical strategy requires detailed molecular studies in well-characterized experimental models combined with careful clinical studies, to identify patient subsets that are likely to respond to this treatment.

Brohlin, M., et al. (2009). "Characterisation of human mesenchymal stem cells following differentiation into Schwann cell-like cells." Neurosci Res **64**(1): 41-49.

Cell-based therapies provide a clinically applicable and available alternative to nerve autografts. Our previous studies have characterised rat-derived mesenchymal stem cells (MSC) and here we have investigated the phenotypic, molecular and functional characteristics of human-derived MSC (hMSC) differentiated along a Schwann cell lineage. The hMSC were isolated from healthy human donors and the identity of the undifferentiated hMSC was confirmed by the detection of MSC specific cells surface markers. The hMSC were differentiated along a glial cell lineage using an established cocktail of growth factors including glial growth factor-2. Following differentiation, the hMSC expressed the key Schwann cell (SC) markers at both the transcriptional and translational level. More importantly, we show the functional effect of hMSC on neurite outgrowth using an in vitro co-culture model system with rat-derived primary sensory neurons. The number of DRG sprouting neurites was significantly enhanced in the presence of differentiated hMSC; neurite length and density (branching) were also increased. These results provide evidence that hMSC can undergo molecular, morphological and functional changes to adopt a SC-like behaviour and, therefore, could be suitable as SC substitutes for nerve repair in clinical applications.

Caja, L., et al. (2011). "The transforming growth factor-beta (TGF-beta) mediates acquisition of a mesenchymal stem cell-like phenotype in human liver cells." J Cell Physiol **226**(5): 1214-1223.

Transforming growth factor-beta (TGF-beta) mediates several and sometime opposite effects in epithelial cells, inducing growth inhibition, and apoptosis but also promoting an epithelial to mesenchymal transition (EMT) process, which enhances cell migration and invasion. TGF-beta plays relevant roles in different liver pathologies; however, very few is known about its specific signaling and cellular effects in human primary hepatocytes. Here we show that TGF-beta inhibits proliferation and induces pro-apoptotic genes (such as BMF or BIM) in primary cultures of human fetal hepatocytes (HFH), but also up-regulates anti-apoptotic genes, such as BCL-XL and XIAP. Inhibition of the epidermal growth factor receptor (EGFR), using gefitinib, abrogates the increase in the expression of the anti-apoptotic genes and significantly enhances cell death. Simultaneously, TGF-beta is able to induce an EMT process in HFH, coincident with Snail up-regulation and a decrease in E-cadherin levels, cells showing mesenchymal proteins and reorganization of the actin cytoskeleton in stress fibers. Interestingly, these cells show loss of expression of specific hepatic genes and increased expression of stem cell markers. Chronic treatment with TGF-beta allows selection of a population of mesenchymal cells with a de-differentiated phenotype, reminiscent of progenitor-like cells. Process is reversible and the mesenchymal stem-like cells re-differentiate to hepatocytes under controlled experimental conditions. In summary, we show for the first time that human hepatocytes may respond to TGF-beta inducing different signals, some of them might contribute to tumor suppression (growth inhibition and apoptosis), but others should mediate liver tumor progression and invasion (EMT and acquisition of a stem-like phenotype).

Cao, H., et al. (2009). "Mesenchymal stem cell-like cells derived from human gastric cancer tissues." Cancer Lett **274**(1): 61-71.

Mesenchymal stem cells (MSCs) have been identified in and isolated from numerous human tissues. The characteristics of mesenchymal stem cells, including their plasticity, the secretion of cytokines, and their low immunogenicity, contribute to their therapeutic potential. It has recently been reported that MSCs are also involved in tumorigenesis and its prognosis. Here, we present the first report of MSC-like cells isolated from human gastric cancer tissues. In our study, gastric cancer-derived MSC-like cells (hGC-MSCs) were isolated from 13 out of 20 cancer tissue samples. Their characteristics, including their morphology, surface antigens, specific gene expression, and differentiation potential, were similar to those of MSCs derived from human bone marrow (hBM-MSCs) but different from gastric cancer cells. The existence of MSC-like cells in gastric cancer tissues suggests that they may be potential targets for cancer therapy and provides an experimental foundation for investigating their role in the initiation and progression of gastric cancers.

Castilho-Fernandes, A., et al. (2011). "Human hepatic stellate cell line (LX-2) exhibits characteristics of bone marrow-derived mesenchymal stem cells." Exp Mol Pathol **91**(3): 664-672.

The LX-2 cell line has characteristics of hepatic stellate cells (HSCs), which are considered pericytes of the hepatic microcirculatory system. Recent studies have suggested that HSCs might have mesenchymal origin. We have performed an extensive characterization of the LX-2 cells and have compared their features with those of mesenchymal cells. Our data show that LX-2 cells have a phenotype resembling activated HSCs as well as bone marrow-derived mesenchymal stem cells (BM-MSCs). Our immunophenotypic analysis showed that LX-2 cells are positive for activated HSC markers (alphaSMA, GFAP, nestin and CD271) and classical mesenchymal makers (CD105, CD44, CD29, CD13, CD90, HLA class-I, CD73, CD49e, CD166 and CD146) but negative for the endothelial marker CD31 and endothelial progenitor cell marker CD133 as well as hematopoietic markers (CD45 and CD34). LX-2 cells also express the same transcripts found in immortalized and primary BM-MSCs (vimentin, annexin 5, collagen 1A, NG2 and CD140b), although at different levels. We show that LX-2 cells are capable to differentiate into multilineage mesenchymal cells in vitro and can stimulate new blood vessel formation in vivo. LX-2 cells appear not to possess tumorigenic potential. Thus, the LX-2 cell line behaves as a multipotent cell line with similarity to BM-MSCs. This line should be useful for further studies to elucidate liver regeneration mechanisms and be the foundation for development of hepatic cell-based therapies.

Chang, J. C., et al. (2013). "Cell orientation and regulation of cell-cell communication in human mesenchymal stem cells on different patterns of electrospun fibers." Biomed Mater **8**(5): 055002.

Cell behavior can be manipulated by the topography of the culture surface. In this study, we examined the intercellular communication and osteogenic differentiation of mesenchymal stem cells (MSCs) grown on electrospun fibers with different orientations and densities. Human bone marrow-derived MSCs (hMSCs) were seeded on poly(epsilon-caprolactone) (PCL) electrospun scaffolds composed of aligned (1D) or cross-aligned (2D) fibers (1.0-1.2 microm diameter) with high, medium, or low fiber densities. It was found that cells preferred to adhere onto electrospun PCL fibers rather than on the flat substrate. The immunofluorescence staining showed that the expression of vinculin, a focal adhesion protein, was limited to the periphery and the two extremities of aligned cells on the edge of the fibers. Electron microscopy showed that cells extended their lamellipodia across the adjacent fibers and proliferated along the direction of fibers. Cells grown on 1D fibrous scaffolds at all fiber densities had an obvious alignment. On 2D fibers, a higher degree of cell alignment was observed at the higher fiber density. On 1D scaffolds, the gap junction intercellular communication (GJIC) quantified by the lucifer yellow dye transfer assay was significantly promoted in the aligned cells in the direction parallel to the fibers but was abolished in the direction perpendicular to the fibers. The expression of osteogenic marker genes (RUNX2, ALP, and OCN) was significantly enhanced in seven days by culture on 1D but not 2D fibers. It was thus proposed that the promoted osteogenic differentiation of hMSCs may be associated with the fiber-guided and directional induction of GJIC.

Chikhovskaya, J. V., et al. (2012). "Human testis-derived embryonic stem cell-like cells are not pluripotent, but possess potential of mesenchymal progenitors." Hum Reprod **27**(1): 210-221.

BACKGROUND: Spontaneous in vitro transition of undifferentiated spermatogonia into the pluripotent cell state has been achieved using neonatal and adult mouse testis tissue. In an effort to establish an analogous source of human patient-specific pluripotent stem cells, several research groups have described the derivation of embryonic stem cell-like cells from primary cultures of human testis. These cells are characterized in all studies as growing in compact colonies, expressing pluripotency-associated markers and possessing multilineage differentiation capabilities in vitro, but only one study claimed their ability to induce teratomas. This controversy initiated a debate about the pluripotent state and origin of human testis-derived ES-like cells (htES-like cells). METHODS: htES-like cell colonies were obtained from primary testicular cultures of three individuals and selectively expanded using culture conditions known to support the propagation of blastocyst-derived human embryonic stem cells (ESCs), mouse epiblast stem cells and 'naive' human ESCs. The stem cell properties of htES-like cells were subsequently assessed by testing the expression of ESC-specific markers, differentiation abilities in vitro and in vivo, and microarray profiling. RESULTS: The expression of pluripotency-associated markers in htES-like cells and their differentiation abilities differed significantly from those of ESCs. Gene expression microarray analysis revealed that htES-like cells possess a transcriptome distinct from human ESCs and fibroblasts, but closely resembling the transcriptome of mesenchymal stem cells (MSCs). The similarity to MSCs was confirmed by detection of SSEA4/CD146 expressing cells within htES-like colonies and efficient in vitro differentiation toward three mesodermal lineages (adipogenic, osteogenic,

chondrogenic). CONCLUSIONS: Taken together, these results indicate that htES-like cells, in contrast to pluripotent stem cells derived from adult mouse testis, are not pluripotent and most likely not of germ cell but of mesenchymal origin.

Chikhovskaya, J. V., et al. (2014). "Mesenchymal origin of multipotent human testis-derived stem cells in human testicular cell cultures." Mol Hum Reprod **20**(2): 155-167.

In contrast to mouse germ cell-derived pluripotent stem cells, the pluripotent state of human testis-derived embryonic stem cell (ESC)-like that spontaneously arise in primary testicular cell cultures remains controversial. Recent studies have shown that these cells closely resemble multipotent mesenchymal stem cells (MSCs), raising the question of their origin and designating these cell populations as multipotent human testis-derived stem cells (mhtSCs) rather than truly ESC-like cells. Here, we evaluate the origin of mhtSCs in vitro by culturing selected testicular cell types. We demonstrate that mhtSCs can be obtained equally efficiently in cultures of pure testicular somatic cells devoid of germ cells. Conversely, cultures with a purified population of germ cells/spermatogonia do not produce any mhtSCs. Based on common molecular characteristics of the somatic starting population and mhtSCs, we conclude that mhtSCs colonies originate from somatic mesenchymal progenitors present in primary testicular cell cultures and do not arise from germ cells undergoing incomplete reprogramming in vitro.

Chistiakova, I. A. and G. G. Polianskaia (2014). "[Influence of human fetal mesenchymal stem cells on glioma cell proliferation. A consequence of cellular crosstalk]." Tsitologiia **56**(11): 800-808.

The effect of mesenchymal stem cells (MSCs) on the growth of various tumors is ambiguous. MSCs derived from different tissues stimulate growth of some tumor types and exert antitumor effects on the other. Several recent reports have shown that crosstalk between tumor cells and MSCs contribute to these effects. The aim of this work was to study the effects of MSCs derived from fetal tissues on the proliferative activity of glioma cells in conditions of prolonged co-cultivation. We have analyzed the proliferative activity of glioma cells exposed to conditioned medium (CM) from MSCs derived from fetal bone marrow (FetMSC) and fetal muscle (M-FetMSC) as well as to CM from co-cultivation of the fetal MSCs with U251MG glioma cells. As a comparison, the influence of CM from adult dermal fibroblasts (DFs) was examined in identical experiments. Using MTT assay, we have found that CM from both the fetal MSCs and adult DFs (without their co-culturing with glioma cells) had no effect on U251MG and A172 glioma cell proliferation. However, CM from early co-cultures (3-9 days) of U251MG cells with FetMSC or M-FetMSC exerted stimulatory effect on U251MG cell proliferation up to 2.3-fold increase, while CM obtained later from the same co-cultures (15-21 days) had inhibitory effect on the proliferation up to arrest of cell division. Analogous experiments with adult DFs have revealed a persistent stimulation of U251MG cell proliferative activity for all 21 days of co-culturing. Immunofluorescence analysis revealed a reduction in the expression of cell cycle protein cyclin D1 in U251MG cells after their treatment with CM taken from 21-days co-cultures of U251MG cells with FetMSC or M-FetMSC. In contrast, CM from 21-days co-cultures of U251MG cells with DFs did not decrease the expression of cyclin D1. These results show that fetal MSCs have dual effect on glioma cell proliferation. In spite of the earlier stimulatory effect on the proliferative activity, prominent inhibition of glioma cell proliferation was observed after three week co-culturing of glioma cells with fetal MSCs. This is the first report demonstrating reversion of tumor cell proliferative program during co-culturing with MSCs for a long time. These data suggest that CM obtained at different time points of cell co-culturing can be used in the modeling of prolonged cellular crosstalk.

Cobo, F., et al. (2008). "Electron microscopy reveals the presence of viruses in mouse embryonic fibroblasts but neither in human embryonic fibroblasts nor in human mesenchymal cells used for hESC maintenance: toward an implementation of microbiological quality assurance program in stem cell banks." Cloning Stem Cells **10**(1): 65-74.

Human embryonic stem cells (hESCs) are expected to open up new avenues in regenerative medicine by allowing the generation of transplantable cells to be used in future cell replacement therapies. Maintenance of hESCs in the presence of xenogenic compounds is likely to prevent their use in future therapeutic applications in humans. Recently, it has been claimed that human foreskin-derived human embryonic fibroblast (HEFs) and human adult marrow cells have the ability to support prolonged expansion of hESCs in culture similar to murine feeders. Here, to minimize the use of xenogenic components for hESC maintenance, we performed transmission electron microscopy-based microbiological studies in an attempt to implement a microbiological Quality Assurance Program in Stem Cell Banks by determining the potential presence of viral particles in MEFs compared with human HEFs and bone marrow-derived mesenchymal cells. We observed in three out of nine MEF samples (33.3%) viruses belonging to the Retroviridae family. Within the Retroviridae family, these viruses have a C morphology, which indicates

they belong to the subfamily Orthoretroviridae. In contrast, no viral particles could be observed in either the HEF samples (n = 5) or the human BM-derived mesenchymal cells (n = 9) analyzed. Based on these experimental microbiological data, we recommend the implementation of microbiological Quality Assurance Programs by means of transmission electron microscopy as a routine technique to assess the potential presence of viral particles in any feeder cell used in stem cell banks and support the use of human cells rather than murine cells as feeders to maintain hESC cultures in an undifferentiated state.

Colletti, E. J., et al. (2006). "The time course of engraftment of human mesenchymal stem cells in fetal heart demonstrates that Purkinje fiber aggregates derive from a single cell and not multi-cell homing." Exp Hematol **34**(7): 926-933.

OBJECTIVE: To study the early time course of engraftment of human mesenchymal stem cells in fetal sheep heart and determine the relative roles of proliferation and homing in formation of aggregates of human Purkinje fiber cells. METHODS: The human sheep xenograft model was utilized for these studies. Prior to injection in the preimmune fetus, human cells were labeled with fluorescent dyes to be able to track human cells at early times of engraftment. RESULTS: Human stem cells were detected in fetal hearts between 29 and 39 hours after intraperitoneal injection. Engraftment was primarily in the Purkinje fiber system. By 45 hours engrafted human cells had a cardiac phenotype. When two groups of human mesenchymal stem cells, each labeled with a different fluorescent dye, were combined prior to injection, aggregates of human Purkinje fiber cells contained cells labeled with either one dye or the other, no aggregate contained cells labeled with both dyes. CONCLUSIONS: Human mesenchymal stem cells introduced into fetal sheep rapidly enter the myocardium. The swift differentiation into a cardiac phenotype indicates that the cardiac milieu has a strong influence on the fate of engrafting human mesenchymal stem cells. The absence of any aggregates of human Purkinje fiber cells containing both fluorescent dyes demonstrates that each aggregate of human Purkinje fiber cells is derived from a single mesenchymal stem cell and not from homing of multiple cells to a hotspot.

Coppi, E., et al. (2007). "ATP modulates cell proliferation and elicits two different electrophysiological responses in human mesenchymal stem cells." Stem Cells **25**(7): 1840-1849.

Bone marrow-derived human mesenchymal stem cells (hMSCs) have the potential to differentiate into several cell lines. Extracellular adenosine 5'-triphosphate (ATP) acts as a potent signaling molecule mediating cell-to-cell communication. Particular interest has been focused in recent years on the role of ATP in stem cell proliferation and differentiation. In the present work, we demonstrate that hMSCs at early stages of culture (P0-P5) spontaneously release ATP, which decreases cell proliferation. Increased hMSC proliferation is induced by the unselective P2 antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonate (PPADS) and by the selective P2Y1 antagonist 2'-deoxy-N6-methyladenosine3',5'-bisphosphate (MRS 2179). A functional role of extracellular ATP in modulating ionic conductances with the whole-cell and/or perforated patch-clamp techniques was also investigated. Exogenous ATP increased both the voltage-sensitive outward and inward currents in 47% of cells, whereas, in 31% of cells, only an increase in inward currents was found. Cells responding in this dual manner to ATP presented different resting membrane potentials. Both ATP-induced effects had varying sensitivity to the P2 antagonists PPADS and MRS 2179. Outward ATP-sensitive currents are carried by potassium ions, since they are blocked by cesium replacement and are Ca2+ -dependent because they are eliminated in the presence of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid. On the basis of different electrophysiological and pharmacological characteristics, we conclude that outward ATP-sensitive currents are due to Ca2+ -dependent K+ -channel activation following stimulation of P2Y receptors, whereas inward ATP-sensitive currents are mediated by P2X receptor activation. In summary, ATP released in early life stages of hMSCs modulates their proliferation rate and likely acts as one of the early factors determining their cell fate. Disclosure of potential conflicts of interest is found at the end of this article.

Corcione, A., et al. (2006). "Human mesenchymal stem cells modulate B-cell functions." Blood **107**(1): 367-372.

Human mesenchymal stem cells (hMSCs) suppress T-cell and dendritic-cell function and represent a promising strategy for cell therapy of autoimmune diseases. Nevertheless, no information is currently available on the effects of hMSCs on B cells, which may have a large impact on the clinical use of these cells. hMSCs isolated from the bone marrow and B cells purified from the peripheral blood of healthy donors were cocultured with different B-cell tropic stimuli. B-cell proliferation was inhibited by hMSCs through an arrest in the G0/G1 phase of the cell cycle and not through the induction of apoptosis. A major mechanism of B-cell suppression was hMSC production of soluble factors, as indicated by transwell experiments. hMSCs inhibited B-cell differentiation

because IgM, IgG, and IgA production was significantly impaired. CXCR4, CXCR5, and CCR7 B-cell expression, as well as chemotaxis to CXCL12, the CXCR4 ligand, and CXCL13, the CXCR5 ligand, were significantly down-regulated by hMSCs, suggesting that these cells affect chemotactic properties of B cells. B-cell costimulatory molecule expression and cytokine production were unaffected by hMSCs. These results further support the potential therapeutic use of hMSCs in immune-mediated disorders, including those in which B cells play a major role.

Costa, M. H. G., et al. (2017). "Tridimensional configurations of human mesenchymal stem/stromal cells to enhance cell paracrine potential towards wound healing processes." J Biotechnol **262**: 28-39.

This study proposes to use alginate encapsulation as a strategy to assess the paracrine activity of 3D- and 2D-cultured human bone marrow mesenchymal stem/stromal cells (BM MSC) in the setting of wound repair and regeneration processes. A side-by-side comparison of MSC culture in three different 3D configurations (spheroids, encapsulated spheroids and encapsulated single cells) versus 2D monolayer cell culture is presented. The results reveal enhanced resistance to oxidative stress and paracrine potential of 3D spheroid-organized BM MSC. MSC spheroids (148+/-2mum diameter) encapsulated in alginate microbeads evidence increased angiogenic and chemotactic potential relatively to encapsulated single cells, as supported by higher secreted levels of angiogenic factors and by functional assays showing the capability of encapsulated MSC to promote formation of tubelike structures and migration of fibroblasts into a wounded area. In addition, a higher expression of the anti-inflammatory factor tumor necrosis factor alpha-induced protein 6 (TSG-6) was demonstrated by RT-PCR for encapsulated and non-encapsulated spheroids. Culture of spheroids within an alginate matrix maintains low aggregation levels below 5% and favors resistance to oxidative stress. These are important factors towards the establishment of more standardized and controlled systems, crucial to explore the paracrine effects of 3D-cultured MSC in therapeutic settings.

Cournil-Henrionnet, C., et al. (2008). "Phenotypic analysis of cell surface markers and gene expression of human mesenchymal stem cells and chondrocytes during monolayer expansion." Biorheology **45**(3-4): 513-526.

Both chondrocytes and mensenchymal stem cells (MSCs) are the most used cell sources for cartilage tissue engineering. However, monolayer expansion to obtain sufficient cells leads to a rapid chondrocyte dedifferentiation and a subsequent ancillary reduced ability of MSCs to differentiate into chondrocytes, thus limiting their application in cartilage repair. The aim of this study was to investigate the influence of the monolayer expansion on the immunophenotype and the gene expression profile of both cell types, and to find the appropriate compromise between monolayer expansion and the remaining chondrogenic characteristics. To this end, human chondrocytes, isolated enzymatically from femoral head slice, and human MSCs, derived from bone marrow, were maintained in monolayer culture up to passage 5. The respective expressions of cell surface markers (CD34, CD45, CD73, CD90, CD105, CD166) and several chondrogenic-related genes for each passage (P0-P5) of those cells were then analyzed using flow cytometry and quantitative real-time PCR, respectively. Flow cytometry analyses showed that, during the monolayer expansion, some qualitative and quantitative regulations occur for the expression of cell surface markers. A rapid increase in mRNA expression of type 1 collagen occurs whereas a significant decrease of type 2 collagen and Sox 9 was observed in chondrocytes through the successive passages. On the other hand, the expansion did not induced obvious change in MSCs gene expression. In conclusion, our results suggest that passage 1 might be the up-limit for chondrocytes in order to achieve their subsequent redifferentiation in 3D scaffold. Nevertheless, MSCs could be expanded in monolayer until passage 5 without loosing their undifferentiated phenotypes.

Crobu, F., et al. (2012). "Differentiation of single cell derived human mesenchymal stem cells into cells with a neuronal phenotype: RNA and microRNA expression profile." Mol Biol Rep **39**(4): 3995-4007.

The adult bone marrow contains a subset of non-haematopoietic cells referred to as bone marrow mesenchymal stem cells (BMSCs). Mesenchymal stem cells (MSCs) have attracted immense research interest in the field of regenerative medicine due to their ability to be cultured for successive passages and multi-lineage differentiation. The molecular mechanisms governing the self-renewal and differentiation of MSCs remain largely unknown. In a previous paper we demonstrated the ability to induce human clonal MSCs to differentiate into cells with a neuronal phenotype (DMSCs). In the present study we evaluated gene expression profiles by Sequential Analysis of Gene Expression (SAGE) and microRNA expression profiles before and after the neuronal differentiation process. Various tissue-specific genes were weakly expressed in MSCs, including those of non-mesodermal origin, suggesting multiple potential tissue-specific differentiation, as well as stemness markers. Expression of OCT4, KLF4 and c-Myc cell reprogramming factors, which are modulated during

the differentiation process, was also observed. Many peculiar nervous tissue genes were expressed at a high level in DMSCs, along with genes related to apoptosis. MicroRNA profiling and correlation with mRNA expression profiles allowed us to identify putative important genes and microRNAs involved in the differentiation of MSCs into neuronal-like cells. The profound difference in gene and microRNA expression patterns between MSCs and DMSCs indicates a real functional change during differentiation from MSCs to DMSCs.

Cronwright, G., et al. (2005). "Cancer/testis antigen expression in human mesenchymal stem cells: down-regulation of SSX impairs cell migration and matrix metalloproteinase 2 expression." Cancer Res **65**(6): 2207-2215.

Several families of genes by and large located on the X chromosome encode proteins of unspecified function. Commonly known as cancer/testis (CT) antigens, they are considered, under normal conditions, only to be expressed in cells of the germ line and placenta. CT genes are also often expressed in cancer cells, hence their classification. Here we report that their expression in normal cells is wider spread and can be observed in cells with the potential for self-renewal and pleuripotency, namely, stem cells. Several CT genes and their products, CT antigens, including SSX, NY-ESO-1, and N-RAGE, were expressed in undifferentiated mesenchymal stem cells (MSCs) and down-regulated after osteocyte and adipocyte differentiation. To elucidate the possible overlapping function played by these genes in cancer and stem cells, a comparative analysis of the localization of their proteins was made. In addition, localization relative to other MSC markers was examined. This revealed that SSX localizes in the cytoplasm and overlap occurs in regions where matrix metalloproteinase 2 (MMP2) and vimentin accumulate. Nevertheless, it was found that no protein interactions between these molecules occur. Further investigation revealed that the migration of a melanoma cell line (DFW), which expresses SSX, MMP2, and vimentin, decreases when SSX is down-regulated. This decrease in cell migration was paralleled by a reduction in MMP2 levels. Analogous to this, SSX expression is down-regulated in MSCs after differentiation; concomitantly a reduction in MMP2 levels occurs. In addition, E-cadherin expression increases, mimicking a mesenchymal epithelial transition. These results afford SSX a functional role in normal stem cell migration and suggest a potentially similar function in cancer cell metastases.

Crop, M. J., et al. (2010). "Human adipose tissue-derived mesenchymal stem cells induce explosive T-cell proliferation." Stem Cells Dev **19**(12): 1843-1853.

Mesenchymal stem cells (MSCs) inhibit the proliferation of allo-activated lymphocytes. This effect is primarily dependent on the secretion of anti-inflammatory factors by MSCs and is enhanced under inflammatory conditions. MSCs, however, also produce factors that can potentially activate resting immune cells. Full understanding of the behavior of MSCs under inflammatory and noninflammatory conditions is crucial when clinical application of MSCs is considered. Human adipose tissue-derived MSCs were cultured with nonactivated peripheral blood mononuclear cells (PBMCs) and the activation, proliferation, and function of PBMCs were examined. Seven days of coculture with autologous or allogeneic MSCs significantly increased the proliferation of PBMCs (3-fold). This effect was observed in both direct and transwell coculture systems. MSCs cocultured with PBMCs showed increased mRNA expression of the proinflammatory mediators interleukin-6 (IL-6), IL-8, tumor necrosis factor-alpha, the growth factors basic fibroblast growth factor and vascular endothelial growth factor-alpha, and the anti-inflammatory factor indoleamine 2,3-dioxygenase. After removal of MSCs, PBMCs showed a spectacular further increase in proliferation, with a maximum of 25-fold after 7 days. This increase in proliferation was not seen when PBMCs were kept in the presence of MSCs. The proliferating fraction of PBMCs largely consisted of CD4(+) T-cells with high CD25 expression and the proportion of CD127(neg)FoxP3(+) regulatory T-cells significantly increased from 5.0% to 8.5% of total CD4(+) T-cells. The expanded T-cells demonstrated normal responses to mitogen or alloantigen stimulation. The CD25(positive) fraction of these cells had immunosuppressive capacity. In conclusion, MSCs can stimulate the activation and proliferation of resting T-cells and generate regulatory T-cells. These findings are important when MSCs are applied in the clinic.

Diederichs, S., et al. (2012). "Interplay between local versus soluble transforming growth factor-beta and fibrin scaffolds: role of cells and impact on human mesenchymal stem cell chondrogenesis." Tissue Eng Part A **18**(11-12): 1140-1150.

Structural extracellular matrix molecules gain increasing attention as scaffolds for cartilage tissue engineering owing to their natural role as a growth factor repository. We recently observed that a collagen-type I/III (Col-I/III) matrix, human recombinant transforming growth factor-beta (TGF-beta) protein, and fibrin hydrogel (FG) combined to a biphasic construct provided sufficient long-term TGF-beta

support to drive in vitro chondrogenesis of human mesenchymal stem cells (hMSC). Here we ask whether FG and Col-I/III can both retain TGF-beta, describe the influence of cell seeding on TGF-beta release, and compare the molecular path of hMSC chondrogenic differentiation under soluble versus local TGF-beta supply. Release of growth factor from scaffolds augmented with increasing amounts of TGF-beta was analyzed over 7 days and chondrogenesis was assessed over 42 days. Low TGF-beta release rates from Col-I/III as opposed to higher release from FG indicated that both molecules retained TGF-beta, with Col-I/III being the superior storage component. Cell seeding enhanced TGF-beta retention in FG by about threefold and almost stopped release beyond 24 h. TGF-beta remained bioactive and supported MSC chondrogenesis without impairing the amount of proteoglycan and collagen-type II deposition per cell and per construct compared to standard scaffold-free MSC pellets supplied with soluble TGF-beta. Local TGF-beta, however, mediated lower cell content, less collagen-type X relative to collagen-type II deposition and no matrix metalloproteinase-13 up-regulation. In conclusion, cells quickly halted release of local TGF-beta from FG, turning FG and Col-I/III into attractive TGF-beta repositories capable to drive full hMSC chondrogenesis, but via a modulated differentiation pathway. Since only part of the changes was reproduced by transient soluble TGF-beta supply, release kinetics alone could not explain the molecular differences, suggesting that local TGF-beta acts distinct from its soluble counterpart.

Diederichs, S. and R. S. Tuan (2014). "Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor." Stem Cells Dev **23**(14): 1594-1610.

Mesenchymal stem cells (MSCs) have a high potential for therapeutic efficacy in treating diverse musculoskeletal injuries and cardiovascular diseases, and for ameliorating the severity of graft-versus-host and autoimmune diseases. While most of these clinical applications require substantial cell quantities, the number of MSCs that can be obtained initially from a single donor is limited. Reports on the derivation of MSC-like cells from pluripotent stem cells (PSCs) are, thus, of interest, as the infinite proliferative capacity of PSCs opens the possibility to generate large amounts of uniform batches of MSCs. However, characterization of such MSC-like cells is currently inadequate, especially with regard to the question of whether these cells are equivalent or identical to MSCs. In this study, we have derived MSC-like cells [induced PSC-derived MSC-like progenitor cells (iMPCs)] using four different methodologies from a newly established induced PSC line reprogrammed from human bone marrow stromal cells (BMSCs), and compared the iMPCs directly with the originating parental BMSCs. The iMPCs exhibited typical MSC/fibroblastic morphology and MSC-typical surface marker profile, and they were capable of differentiation in vitro along the osteogenic, chondrogenic, and adipogenic lineages. However, compared with the parental BMSCs, iMPCs displayed a unique expression pattern of mesenchymal and pluripotency genes and were less responsive to traditional BMSC differentiation protocols. We, therefore, conclude that iMPCs generated from PSCs via spontaneous differentiation represent a distinct population of cells which exhibit MSC-like characteristics.

Diez, J. M., et al. (2015). "Culture of human mesenchymal stem cells using a candidate pharmaceutical grade xeno-free cell culture supplement derived from industrial human plasma pools." Stem Cell Res Ther **6**: 28.

INTRODUCTION: Fetal bovine serum (FBS) is an animal product used as a medium supplement. The animal origin of FBS is a concern if cultured stem cells are to be utilized for human cell therapy. Therefore, a substitute for FBS is desirable. In this study, an industrial, xeno-free, pharmaceutical-grade supplement for cell culture (SCC) under development at Grifols was tested for growth of human mesenchymal stem cells (hMSCs), cell characterization, and differentiation capacity. METHODS: SCC is a freeze-dried product obtained through cold-ethanol fractionation of industrial human plasma pools from healthy donors. Bone marrow-derived hMSC cell lines were obtained from two commercial suppliers. Cell growth was evaluated by culturing hMSCs with commercial media or media supplemented with SCC or FBS. Cell viability and cell yield were assessed with an automated cell counter. Cell surface markers were studied by indirect immunofluorescence assay. Cells were cultured then differentiated into adipocytes, chondrocytes, osteoblasts, and neurons, as assessed by specific staining and microscopy observation. RESULTS: SCC supported the growth of commercial hMSCs. Starting from the same number of seeded cells in two consecutive passages of culture with medium supplemented with SCC, hMSC yield and cell population doubling time were equivalent to the values obtained with the commercial medium and was consistent among lots. The viability of hMSCs was higher than 90%, while maintaining the characteristic phenotype of undifferentiated hMSCs (positive for CD29, CD44, CD90, CD105, CD146, CD166 and Stro-1; negative for CD14 and CD19). Cultured hMSCs maintained the potential for differentiation into adipocytes, chondrocytes, osteoblasts, and neurons.

CONCLUSIONS: The tested human plasma-derived SCC sustains the adequate growth of hMSCs, while preserving their differentiation capacity. SCC can be a potential candidate for cell culture supplement in advanced cell therapies.

Ding, D. C., et al. (2015). "Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy." Cell Transplant **24**(3): 339-347.

The human umbilical cord is a promising source of mesenchymal stem cells (HUCMSCs). Unlike bone marrow stem cells, HUCMSCs have a painless collection procedure and faster self-renewal properties. Different derivation protocols may provide different amounts and populations of stem cells. Stem cell populations have also been reported in other compartments of the umbilical cord, such as the cord lining, perivascular tissue, and Wharton's jelly. HUCMSCs are noncontroversial sources compared to embryonic stem cells. They can differentiate into the three germ layers that promote tissue repair and modulate immune responses and anticancer properties. Thus, they are attractive autologous or allogenic agents for the treatment of malignant and nonmalignant solid and soft cancers. HUCMCs also can be the feeder layer for embryonic stem cells or other pluripotent stem cells. Regarding their therapeutic value, storage banking system and protocols should be established immediately. This review critically evaluates their therapeutic value, challenges, and future directions for their clinical applications.

Dolatshahi-Pirouz, A., et al. (2014). "A combinatorial cell-laden gel microarray for inducing osteogenic differentiation of human mesenchymal stem cells." Sci Rep **4**: 3896.

Development of three dimensional (3D) microenvironments that direct stem cell differentiation into functional cell types remains a major challenge in the field of regenerative medicine. Here, we describe a new platform to address this challenge by utilizing a robotic microarray spotter for testing stem cell fates inside various miniaturized cell-laden gels in a systematic manner. To demonstrate the feasibility of our platform, we evaluated the osteogenic differentiation of human mesenchymal stem cells (hMSCs) within combinatorial 3D niches. We were able to identify specific combinations, that enhanced the expression of osteogenic markers. Notably, these 'hit' combinations directed hMSCs to form mineralized tissue when conditions were translated to 3D macroscale hydrogels, indicating that the miniaturization of the experimental system did not alter stem cell fate. Overall, our findings confirmed that the 3D cell-laden gel microarray can be used for screening of different conditions in a rapid, cost-effective, and multiplexed manner for a broad range of tissue engineering applications.

Domev, H., et al. (2012). "Efficient engineering of vascularized ectopic bone from human embryonic stem cell-derived mesenchymal stem cells." Tissue Eng Part A **18**(21-22): 2290-2302.

Human mesenchymal stem cells (hMSCs) can be derived from various adult and fetal tissues. However, the quality of tissues for the isolation of adult and fetal hMSCs is donor dependent with a nonreproducible yield. In addition, tissue engineering and cell therapy require large-scale production of a pure population of lineage-restricted stem cells that can be easily induced to differentiate into a specific cell type. Therefore, human embryonic stem cells (hESCs) can provide an alternative, plentiful source for generation of reproducible hMSCs. We have developed efficient differentiation protocols for derivation of hMSCs from hESCs, including coculture with murine OP9 stromal cells and feeder layer-free system. Our protocols have resulted in the generation of up to 49% of hMSCs, which expressed CD105, CD90, CD29, and CD44. The hMSCs exhibited high adipogenic, chondrocytic, and osteogenic differentiation in vitro. The latter correlated with osteocalcin secretion and vascular endothelial growth factor (VEGF) production by the differentiating hMSCs. hMSC-derived osteoblasts further differentiated and formed ectopic bone in vivo, and induced the formation of blood vessels in Matrigel implants. Our protocol enables generation of a purified population of hESC-derived MSCs, with the potential of differentiating into several mesodermal lineages, and particularly into vasculogenesis-inducing osteoblasts, which can contribute to the development of bone repair protocols.

Dos Santos, F., et al. (2010). "Ex vivo expansion of human mesenchymal stem cells: a more effective cell proliferation kinetics and metabolism under hypoxia." J Cell Physiol **223**(1): 27-35.

The low bone marrow (BM) MSC titers demand a fast ex vivo expansion process to meet the clinically relevant cell dosage. Attending to the low oxygen tension of BM in vivo, we studied the influence of hypoxia on human BM MSC proliferation kinetics and metabolism. Human BM MSC cultured under 2% (hypoxia) and 20% O(2) (normoxia) were characterized in terms of proliferation, cell division kinetics and metabolic patterns. BM MSC cultures under hypoxia displayed an early start of the exponential growth phase, and cell numbers obtained at each time point throughout culture were consistently higher under low O(2), resulting in a higher fold increase after 12 days under hypoxia (40 +/- 10 vs. 30 +/- 6). Cell labeling with PKH26 allowed us to determine that after 2 days

of culture, a significant higher cell number was already actively dividing under 2% compared to 20% O(2) and BM MSC expanded under low oxygen tension displayed consistently higher percentages of cells in the latest generations (generations 4-6) until the 5th day of culture. Cells under low O(2) presented higher specific consumption of nutrients, especially early in culture, but with lower specific production of inhibitory metabolites. Moreover, 2% O(2) favored CFU-F expansion, while maintaining BM MSC characteristic immunophenotype and differentiative potential. Our results demonstrated a more efficient BM MSC expansion at 2% O(2), compared to normoxic conditions, associated to an earlier start of cellular division and supported by an increase in cellular metabolism efficiency towards the maximization of cell yield for application in clinical settings.

Dozza, B., et al. (2012). "Cell growth inhibition and apoptotic effect of the rexinoid 6-OH-11-O-hydroxyphenantrene on human osteosarcoma and mesenchymal stem cells." Toxicol In Vitro **26**(1): 142-149.

Natural derivatives of vitamin A, including all-trans-retinoic acid (ATRA), commonly known as retinoids, currently produce favorable results in the treatment of many types of tumors. The rexinoid 6-OH-11-O-hydroxyphenantrene (IIF) is a synthetic derivative of ATRA. Previous in vitro and in vivo studies demonstrated that IIF is able to induce growth inhibition of various cancer cells and is a potent apoptosis-inducing agent with clinical potential. Osteosarcoma (OS) is the most common type of bone cancer, characterized by a rising aggressiveness. Recent evidences suggest that mesenchymal stem cells (MSC) may favour tumor growth and progression. Thus, it is important to investigate whether a compound with potential anti-tumoral properties such as IIF affects not only tumor cells but also MSC. The current study is an attempt to understand the mode of the potential cytotoxicity of IIF on OS cells and MSC. The response to IIF treatment of osteosarcoma SaOS-2, MG63, and U2OS cells and of bone marrow-derived MSC was the subject of investigation. The results showed that IIF significantly inhibited cell growth in OS cell lines and MSC in both a time- and dose-dependent manner, as evaluated by methylene blue assay. This was also associated with altered cell morphology and an increase in cell death with the involvement of apoptosis as demonstrated by NucleoCounter, Hoechst 33342 staining and FACS analysis. No cell death and apoptosis was found in U2OS cells. Analysis of cells treated with 20 and 40muM IIF for 24h by western blot suggests the activation of initiator caspase 9, indicating the involvement of caspases in inducing apoptosis. Furthermore, IIF upregulated the expression of the pro-apoptotic protein Bax and downregulated the anti-apoptotic protein Bcl2. For the first time, our results collectively provide an evidence for cell growth inhibition and activation of apoptosis in human OS cells and MSC by IIF. These results confirm that IIF may be an effective compound for anticancer treatment, including that of OS.

Dregalla, R. C., et al. (2014). "Amide-type local anesthetics and human mesenchymal stem cells: clinical implications for stem cell therapy." Stem Cells Transl Med **3**(3): 365-374.

In the realm of regenerative medicine, human mesenchymal stem cells (hMSCs) are gaining attention as a cell source for the repair and regeneration of tissues spanning an array of medical disciplines. In orthopedics, hMSCs are often delivered in a site-specific manner at the area of interest and may require the concurrent application of local anesthetics (LAs). To address the implications of using hMSCs in combination with anesthetics for intra-articular applications, we investigated the effect that clinically relevant doses of amide-type LAs have on the viability of bone marrow-derived hMSCs and began to characterize the mechanism of LA-induced hMSC death. In our study, culture-expanded hMSCs from three donors were exposed to the amide-type LAs ropivacaine, lidocaine, bupivacaine, and mepivacaine. To replicate the physiological dilution of LAs once injected into the synovial capsule, each anesthetic was reduced to 12.5%, 25%, and 50% of the stock solution and incubated with each hMSC line for 40 minutes, 120 minutes, 360 minutes, and 24 hours. At each time point, cell viability assays were performed. We found that extended treatment with LAs for 24 hours had a significant impact on both hMSC viability and adhesion. In addition, hMSC treatment with three of the four anesthetics resulted in cell death via apoptosis following brief exposures. Ultimately, we concluded that amide-type LAs induce hMSC apoptosis in a time- and dose-dependent manner that may threaten clinical outcomes, following a similar trend that has been established between these particular anesthetics and articular chondrocytes both in vitro and in vivo.

English, K., et al. (2009). "Cell contact, prostaglandin E(2) and transforming growth factor beta 1 play non-redundant roles in human mesenchymal stem cell induction of CD4+CD25(High) forkhead box P3+ regulatory T cells." Clin Exp Immunol **156**(1): 149-160.

Adult human mesenchymal stromal or stem cells (MSC) can differentiate into a variety of cell types and are candidate cellular therapeutics in regenerative medicine. Surprisingly, these cells also display multiple potent immunomodulatory capabilities, including

allosuppression, making allogeneic cell therapy a possibility. The exact mechanisms involved in regulatory T cell induction by allogeneic human MSC was examined, using purified CD4+ populations and well-characterized bone marrow-derived adult human MSC. Allogeneic MSC were shown to induce forkhead box P3 (FoxP3)+ and CD25+ mRNA and protein expression in CD4+ T cells. This phenomenon required direct contact between MSC and purified T cells, although cell contact was not required for MSC induction of FoxP3 expression in an unseparated mononuclear cell population. In addition, through use of antagonists and neutralizing antibodies, MSC-derived prostaglandins and transforming growth factor (TGF)-beta1 were shown to have a non-redundant role in the induction of CD4+CD25+FoxP3+ T cells. Purified CD4+CD25+ T cells induced by MSC co-culture expressed TGF-beta1 and were able to suppress alloantigen-driven proliferative responses in mixed lymphocyte reaction. These data clarify the mechanisms of human MSC-mediated allosuppression, supporting a sequential process of regulatory T cell induction involving direct MSC contact with CD4+ cells followed by both prostaglandin E(2) and TGF-beta1 expression. Overall, this study provides a rational basis for ongoing clinical studies involving allogeneic MSC.

Eyckmans, J., et al. (2012). "Adhesive and mechanical regulation of mesenchymal stem cell differentiation in human bone marrow and periosteum-derived progenitor cells." Biol Open **1**(11): 1058-1068.

It has previously been demonstrated that cell shape can influence commitment of human bone marrow-derived mesenchymal stem cells (hBMCs) to adipogenic, osteogenic, chondrogenic, and other lineages. Human periosteum-derived cells (hPDCs) exhibit multipotency similar to hBMCs, but hPDCs may offer enhanced potential for osteogenesis and chondrogenesis given their apparent endogenous role in bone and cartilage repair in vivo. Here, we examined whether hPDC differentiation is regulated by adhesive and mechanical cues comparable to that reported for hBMC differentiation. When cultured in the appropriate induction media, hPDCs at high cell seeding density demonstrated enhanced levels of adipogenic or chondrogenic markers as compared with hPDCs at low cell seeding density. Cell seeding density correlated inversely with projected area of cell spreading, and directly limiting cell spreading with micropatterned substrates promoted adipogenesis or chondrogenesis while substrates promoting cell spreading supported osteogenesis. Interestingly, cell seeding density influenced differentiation through both changes in cell shape and non-shape-mediated effects: density-dependent adipogenesis and chondrogenesis were regulated primarily by cell shape whereas non-shape effects strongly influenced osteogenic potential. Inhibition of cytoskeletal contractility by adding the Rho kinase inhibitor Y27632 further enhanced adipogenic differentiation and discouraged osteogenic differentiation of hPDCs. Together, our results suggest that multipotent lineage decisions of hPDCs are impacted by cell adhesive and mechanical cues, though to different extents than hBMCs. Thus, future studies of hPDCs and other primary stem cell populations with clinical potential should consider varying biophysical metrics for more thorough optimization of stem cell differentiation.

Fonseka, M., et al. (2012). "Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSC) inhibit the proliferation of K562 (human erythromyeloblastoid leukaemic cell line)." Cell Biol Int **36**(9): 793-801.

hUCB-MSC (human umbilical cord blood-derived mesenchymal stem cells) offer an attractive alternative to bone marrow-derived MSC for cell-based therapy by being less invasive a source of biological material. We have evaluated the effect of hUCB-MSC on the proliferation of K562 (an erythromyeloblastoid cell line) and the cytokine secretion pattern of hUCB-MSC. Co-culturing of hUCB-MSC and K562 resulted in inhibition of proliferation of K562 in a dose-dependent manner. However, the anti-proliferative effect was reduced in transwells, suggesting the importance of direct cell-to-cell contact. hUCB-MSC inhibited proliferation of K562, arresting them in the G0 /G1 phase. NO (nitric oxide) was not involved in the hUCB-MSC-mediated tumour suppression. The presence of IL-6 (interleukin 6) and IL-8 were obvious in the hUCB-MSC conditioned media, but no significant increase was found in 29 other cytokines. Th1 cytokines, IFNalpha (interferon alpha), Th2 cytokine IL-4 and Th17 cytokine, IL-17 were not secreted by hUCB-MSC. There was an increase in the number of hUCB-MSC expressing the latent membrane-bound form of TGFbeta1 co-cultured with K562. The anti-proliferative effect of hUCB-MSC was due to arrest of the growth of K562 in the G0 /G1 phase. The mechanisms underlying increased IL-6 and IL-8 secretion and LAP (latency-associated peptide; TGFbeta1) by hUCB-MSC remains unknown.

Fossett, E., et al. (2012). "The effects of ageing on proliferation potential, differentiation potential and cell surface characterisation of human mesenchymal stem cells." Curr Stem Cell Res Ther **7**(4): 282-286.

Mesenchymal stem cells (MSCs) have a great capacity for use in regenerative medicine and other clinical applications. However, one question creating curiosity of their use, is how they are affected by

ageing. As we now live within an ageing population, the prevalence of age related disorders is increasing, so it is important to investigate how effectively MSCs from older patients can be expanded and differentiated in vitro before their use in autologous cell transplantation. This paper will look at how ageing effects proliferation potential, differentiation potential and cell surface characterisation of human mesenchymal stem cells.

Freida, D., et al. (2013). "Human bone marrow mesenchymal stem cells regulate biased DNA segregation in response to cell adhesion asymmetry." Cell Rep **5**(3): 601-610.

Biased DNA segregation is a mitotic event in which the chromatids carrying the original template DNA strands and those carrying the template copies are not segregated randomly into the two daughter cells. Biased segregation has been observed in several cell types, but not in human mesenchymal stem cells (hMSCs), and the factors affecting this bias have yet to be identified. Here, we have investigated cell adhesion geometries as a potential parameter by plating hMSCs from healthy donors on fibronectin-coated micropatterns. On symmetric micropatterns, the segregation of sister chromatids to the daughter cells appeared random. In contrast, on asymmetric micropatterns, the segregation was biased. This sensitivity to asymmetric extracellular cues was reproducible in cells from all donors but was not observed in human skin-derived fibroblasts or in a fibroblastic cell line used as controls. We conclude that the asymmetry of cell adhesion is a major factor in the regulation of biased DNA segregation in hMSCs.

Friedman, R., et al. (2007). "Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation." Biol Blood Marrow Transplant **13**(12): 1477-1486.

The Wharton's jelly of the umbilical cord is rich in mesenchymal stem cells (UC-MSCs) that fulfill the criteria for MSCs. Here we describe a novel, simple method of obtaining and cryopreserving UC-MSCs by extracting the Wharton's jelly from a small piece of cord, followed by mincing the tissue and cryopreserving it in autologous cord plasma to prevent exposure to allogeneic or animal serum. This direct freezing of cord microparticles without previous culture expansion allows the processing and freezing of umbilical cord blood (UCB) and UC-MSCs from the same individual on the same day on arrival in the laboratory. UC-MSCs produce significant concentrations of hematopoietic growth factors in culture and augment hematopoietic colony formation when co-cultured with UCB mononuclear cells. Mice undergoing transplantation with limited numbers of human UCB cells or CD34(+) selected cells demonstrated augmented engraftment when UC-MSCs were co-transplanted. We also explored whether UC-MSCs could be further manipulated by transfection with plasmid-based vectors. Electroporation was used to introduce cDNA and mRNA constructs for GFP into the UC-MSCs. Transfection efficiency was 31% for cDNA and 90% for mRNA. These data show that UC-MSCs represent a reliable, easily accessible, noncontroversial source of MSCs. They can be prepared and cryopreserved under good manufacturing practices (GMP) conditions and are able to enhance human hematopoietic engraftment in SCID mice. Considering their cytokine production and their ability to be easily transfected with plasmid-based vectors, these cells should have broad applicability in human cell-based therapies.

Fu, Q., et al. (2017). "Human decidua mesenchymal stem cells regulate decidual natural killer cell function via interactions between collagen and leukocyteassociated immunoglobulinlike receptor 1." Mol Med Rep **16**(3): 2791-2798.

The development of maternal tolerance to the fetal allograft in critical for the maintenance of the pregnancy, and it is accompanied by the development of a special decidual natural killer (dNK) cell tolerance phenotype. To understand the factors that influence dNK cells during early pregnancy, the present study aimed to identify mesenchymal stem cells (MSCs) from human firsttrimester deciduas, termed decidual MSCs (DMSCs), and to investigate the effect of DMSCs on the regulation of dNK cells via collagen. Decidual samples were collected from women with normal pregnancy that had undergone elective vaginal surgical terminations at 69 weeks gestation. DMSCs derived from human decidual tissues were cultured under differentiation conditions to examine their multipotent differentiation capacities, and the expression of MSCspecific markers, including cluster of differentiation (CD)44, CD73, CD105, CD90, CD34, CD31, CD14, CD45, CD11b and human leukocyte antigenantigen D related, was determined. dNK cells were cocultured with DMSCs in order to examine the effect of DMSCs on the tolerance phenotype of dNK cells. The expression of cell surface molecules, natural cytotoxicity triggering receptor 3 and killer cell immunoglobulinlike receptor (KIR) 2DL1, and the secretion of cytokines, including interferongamma, tumor necrosis factor (TNF)alpha, interleukin (IL)10, IL4 and perforin, were examined by flow cytometry analysis. To determine whether the regulation of dNK cells by DMSCs was mediated by collagen, DMSCs were pretreated with human recombinant leukocyteassociated immunoglobulinlike receptor (LAIR)2 and transfected with pScoRGFPhP4H to

inhibit the interaction between LAIR1 and collagen. The present results demonstrated that collagen produced by DMSCs increased the expression of KIR2DL1 and IL4, decrease the expression of NKp30 and TNFalpha. In conclusion, the results of the present study demonstrated that DMSCs may be cultured in vitro for prolonged periods, whilst retaining the ability to differentiate into different cell lineages. In addition, DMSCs may modulate the function of dNK cells via the interaction between collagen and LAIR1.

Fu, Q. L., et al. (2012). "Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis." Allergy **67**(10): 1215-1222.

BACKGROUND: Human induced pluripotent stem cells (iPSCs) possess remarkable self-renewal capacity and the potential to differentiate into novel cell types, such as mesenchymal stem cells (MSCs). iPSC-MSCs have been shown to enhance tissue regeneration and attenuate tissue ischaemia; however, their contribution to the immune regulation of Th2-skewed allergic rhinitis (AR) and asthma remains unclear. OBJECTIVE: This study compared the immunomodulatory effects of iPSC-MSCs and bone marrow-derived MSCs (BM-MSCs) on lymphocyte proliferation, T-cell phenotypes and cytokine production in peripheral blood mononuclear cells (PBMCs) in patients with AR, and investigated the possible molecular mechanisms underlying the immunomodulatory properties of iPSC-MSCs. METHODS: In co-cultures of PBMCs with iPSC-MSCs or BM-MSCs, lymphocyte proliferation was evaluated using 3H-thymidine (3H-TdR) uptake, carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) assays; the regulatory T-cell (Treg) phenotype was determined by flow cytometry, and cytokine levels were measured using an enzyme-linked immunosorbent assay. The immunomodulatory properties of both MSCs were further evaluated using NS398 and transwell experiments. RESULTS: Similar to BM-MSCs, we determined that iPSC-MSCs significantly inhibit lymphocyte proliferation and promote Treg response in PBMCs ($P < 0.05$). Accordingly, the cytokine milieu (IFN-gamma, IL-4, IL-5, IL-10 and IL-13) in the supernatants of PBMCs changed significantly ($P < 0.05$). The immunomodulatory properties of iPSC-MSCs and BM-MSCs were associated with prostaglandin E2 (PGE2) production and cell-cell contact. CONCLUSIONS: These data demonstrate that iPSC-MSCs are capable of modulating T-cell phenotypes towards Th2 suppression through inducing Treg expansion, suggesting that iPSC-MSCs can be used as an alternative candidate to adult MSCs to treat allergic airway diseases.

Fukuchi, Y., et al. (2004). "Human placenta-derived cells have mesenchymal stem/progenitor cell potential." Stem Cells **22**(5): 649-658.

Mesenchymal stem/progenitor cells (MSCs) are widely distributed in a variety of tissues in the adult human body (e.g., bone marrow [BM], kidney, lung, and liver). These cells are also present in the fetal environment (e.g., blood, liver, BM, and kidney). However, MSCs are a rare population in these tissues. Here we tried to identify cells with MSC-like potency in human placenta. We isolated adherent cells from trypsin-digested term placentas and established two clones by limiting dilution. We examined these cells for morphology, surface markers, gene expression patterns, and differentiation potential and found that they expressed several stem cell markers, hematopoietic/ endothelial cell-related genes, and organ-specific genes, as determined by reverse transcription-polymerase chain reaction and fluorescence-activated cell sorter analysis. They also showed osteogenic and adipogenic differentiation potentials under appropriate conditions. We suggest that placenta-derived cells have multilineage differentiation potential similar to MSCs in terms of morphology, cell-surface antigen expression, and gene expression patterns. The placenta may prove to be a useful source of MSCs.

Gadkari, R., et al. (2014). "Human embryonic stem cell derived-mesenchymal stem cells: an alternative mesenchymal stem cell source for regenerative medicine therapy." Regen Med **9**(4): 453-465.

AIM: To enumerate and characterize mesenchymal stem cells (MSC) derived from human embryonic stem cells (hESC) for clinical application. MATERIALS & METHODS: hESC were differentiated into hESC-MSC and characterized by the expression of surface markers using flow cytometry. hESC-MSC were evaluated with respect to growth kinetics, colony-forming potential, as well as osteogenic and adipogenic differentiation capacity. Immunosuppressive effects were assessed using peripheral blood mononuclear cell (PBMC) proliferation and cytotoxicity assays. RESULTS: hESC-MSC showed similar morphology, and cell surface markers as adipose (AMSC) and bone marrow-derived MSC (BMSC). hESC-MSC exhibited a higher growth rate during early in vitro expansion and equivalent adipogenic and osteogenic differentiation and colony-forming potential as AMSC and BMSC. hESC-MSC demonstrated similar immunosuppressive effects as AMSC and BMSC. CONCLUSION: hESC-MSC were comparable to BMSC and AMSC and hence can be used as an alternative source of MSC for clinical applications.

Gaebel, R., et al. (2011). "Cell origin of human mesenchymal stem cells determines a different healing performance in cardiac regeneration." PLoS One **6**(2): e15652.

The possible different therapeutic efficacy of human mesenchymal stem cells (hMSC) derived from umbilical cord blood (CB), adipose tissue (AT) or bone marrow (BM) for the treatment of myocardial infarction (MI) remains unexplored. This study was to assess the regenerative potential of hMSC from different origins and to evaluate the role of CD105 in cardiac regeneration. Male SCID mice underwent LAD-ligation and received the respective cell type (400.000/per animal) intramyocardially. Six weeks post infarction, cardiac catheterization showed significant preservation of left ventricular functions in BM and CD105(+)-CB treated groups compared to CB and nontreated MI group (MI-C). Cell survival analyzed by quantitative real time PCR for human GAPDH and capillary density measured by immunostaining showed consistent results. Furthermore, cardiac remodeling can be significantly attenuated by BM-hMSC compared to MI-C. Under hypoxic conditions in vitro, remarkably increased extracellular acidification and apoptosis has been detected from CB-hMSC compared to BM and CD105 purified CB-derived hMSC. Our findings suggests that hMSC originating from different sources showed a different healing performance in cardiac regeneration and CD105(+) hMSC exhibited a favorable survival pattern in infarcted hearts, which translates into a more robust preservation of cardiac function.

Gagari, E., et al. (2006). "Expression of stem cell factor and its receptor, c-kit, in human oral mesenchymal cells." Eur J Oral Sci **114**(5): 409-415.

Stem cell factor (SCF) is the pleiotropic ligand for the tyrosine kinase receptor, c-kit. Ligand and receptor are usually expressed in different cell types, and binding of SCF to c-kit promotes cell proliferation, differentiation, and recruitment of progenitor cells in various biologic systems. However, the localization of these two molecules in cells of the oral cavity has not been systematically examined. We investigated the expression of SCF and c-kit in human dental pulp (HDP) cells as well as in human gingival fibroblasts (HGF). Both alternatively spliced isoforms of SCF were detected (through reverse transcription-polymerase chain reaction) in RNA obtained from the two cell types. Western analysis established that both cell types express SCF and/or c-kit, whereas flow cytometry demonstrated distinct cell populations expressing only the ligand (SCF), only the receptor (c-kit), or co-expressing the two. HDP cultures showed higher soluble SCF (sSCF) production associated with faster cell growth, as compared with HGF cultures. In both cell types, however, sSCF levels appeared to increase as a result of in vitro aging and/or differentiation.

Galindo, S., et al. (2017). "Therapeutic Effect of Human Adipose Tissue-Derived Mesenchymal Stem Cells in Experimental Corneal Failure Due to Limbal Stem Cell Niche Damage." Stem Cells **35**(10): 2160-2174.

Limbal stem cells are responsible for the continuous renewal of the corneal epithelium. The destruction or dysfunction of these stem cells or their niche induces limbal stem cell deficiency (LSCD) leading to visual loss, chronic pain, and inflammation of the ocular surface. To restore the ocular surface in cases of bilateral LSCD, an extraocular source of stem cells is needed to avoid dependence on allogeneic limbal stem cells that are difficult to obtain, isolate, and culture. The aim of this work was to test the tolerance and the efficacy of human adipose tissue-derived mesenchymal stem cells (hAT-MSCs) to regenerate the ocular surface in two experimental models of LSCD that closely resemble different severity grades of the human pathology. hAT-MSCs transplanted to the ocular surface of the partial and total LSCD models developed in rabbits were well tolerated, migrated to inflamed tissues, reduced inflammation, and restrained the evolution of corneal neovascularization and corneal opacity. The expression profile of the corneal epithelial cell markers CK3 and E-cadherin, and the limbal epithelial cell markers CK15 and p63 was lost in the LSCD models, but was partially recovered after hAT-MSC transplantation. For the first time, we demonstrated that hAT-MSCs improve corneal and limbal epithelial phenotypes in animal LSCD models. These results support the potential use of hAT-MSCs as a novel treatment of ocular surface failure due to LSCD. hAT-MSCs represent an available, non-immunogenic source of stem cells that may provide therapeutic benefits in addition to reduce health care expenses. Stem Cells 2017;35:2160-2174.

Ghannam, S., et al. (2010). "Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype." J Immunol **185**(1): 302-312.

Mesenchymal stem cells (MSCs) exert immunomodulatory properties via the inhibition of T cell activation and proliferation. Because of the deleterious role of Th17 cells in the pathogenesis of inflammatory disease, we investigated whether proinflammatory cytokines could modify the expression of adhesion molecules on human MSCs, thereby contributing to increased Th17 cell adhesion to MSCs and, as a consequence, modulating the function of the latter cells. IFN-gamma and TNF-alpha

synergistically enhanced the expression of CD54 by MSCs, enabling the CCR6 chemokine ligand CCL20 to induce in vitro adhesion of Th17 cells to MSCs. MSCs prevented the in vitro differentiation of naive CD4(+) T cells into Th17 cells and inhibited the production of IL-17, IL-22, IFN-gamma, and TNF-alpha by fully differentiated Th17 cells; this was mediated, in part, via PGE(2), the production of which was enhanced in cocultures with Th17 cells. Moreover, MSCs induced the production of IL-10 and trimethylation of histone H3K4me3 at the promoter of the FOXP3 gene locus, whereas it suppressed trimethylation of the corresponding region in the RORC gene in Th17 cells. These epigenetic changes were associated with the induction of fork head box p3 and the acquisition by Th17 cells of the capacity to inhibit in vitro proliferative responses of activated CD4(+) T cells, which was enhanced when MSCs were preincubated with IFN-gamma and TNF-alpha. These results showed that, under inflammatory conditions, MSCs mediate the adhesion of Th17 cells via CCR6 and exert anti-inflammatory effects through the induction of a T cell regulatory phenotype in these cells.

Gildehaus, F. J., et al. (2011). "Impact of indium-111 oxine labelling on viability of human mesenchymal stem cells in vitro, and 3D cell-tracking using SPECT/CT in vivo." Mol Imaging Biol **13**(6): 1204-1214.

PURPOSE: This study investigates the effects of (111)In-oxine incorporation on human mesenchymal stem cells' (hMSC) biology and viability, and the applicability of (111)In-oxine for single-photon emission computed tomography/X-ray computed tomography (SPECT/CT) monitoring of hMSC in vivo. PROCEDURES: HMSC were labelled with 10 Bq/cell. Cellular retention of radioactivity, cell survival, and migration were evaluated over 48 h. Metabolic activity was assessed over 14 days and the hMSC's stem cell character was evaluated. Serial SPECT/CT was performed after intra-osseous injection to athymic rats over 48 h. RESULTS: Labelling efficiency was 25%, with 61% of incorporated (111)In remaining in the hMSC at 48 h. The radiolabelling was without effect on cell viability, stem cell character, and plasticity, whereas metabolic activity and migration were significantly reduced. Grafted cells could be imaged in situ with SPECT/CT. CONCLUSIONS: (111)In-oxine labelling moderately impaired hMSC's functional integrity while preserving their stem cell character. Combined SPECT/CT imaging of (111)In-oxine-labelled hMSC opens the possibility for non-invasive sequential monitoring of therapeutic stem cells.

Gindraux, F., et al. (2007). "Human and rodent bone marrow mesenchymal stem cells that express primitive stem cell markers can be directly enriched by using the CD49a molecule." Cell Tissue Res **327**(3): 471-483.

Bone marrow (BM) from human and rodent species contains a population of multipotential cells referred to as mesenchymal stem cells (MSCs). Currently, MSCs are isolated indirectly by using a culture step and then the generation of fibroblast colony-forming units (CFU-fs). Unprocessed or native BM MSCs have not yet been fully characterised. We have previously developed a direct enrichment method for the isolation of MSCs from human BM by using the CD49a protein (alpha1-integrin subunit). As the CD49a gene is highly conserved in mammals, we have evaluated whether this direct enrichment can be employed for BM cells from rodent strains (rat and mouse). We have also studied the native phenotype by using both immunodetection and immunomagnetic methods and have compared MSCs from mouse, rat and human BM. As is the case for human BM, we have demonstrated that all rodent multipotential CFU-fs are contained within the CD49a-positive cell population. However, in the mouse, the number of CFU-fs is strain-dependent. Interestingly, all rat and mouse Sca-1-positive cells are concentrated within the CD49a-positive fraction and also contain all CFU-fs. In human, the colonies have been detected in the CD49a/CD133 double-positive population. Thus, the CD49a protein is a conserved marker that permits the direct enrichment of BM MSCs from various mammalian species; these cells have been phenotyped as true BM stem cells.

Giuliani, M., et al. (2011). "Human mesenchymal stem cells derived from induced pluripotent stem cells down-regulate NK-cell cytolytic machinery." Blood **118**(12): 3254-3262.

A major issue in immunosuppressive biotherapy is the use of mesenchymal stem cells (MSCs) that harbor regulatory capacity. However, currently used bone marrow-derived MSCs (BM-MSCs) are short-lived and cannot assure long lasting immunoregulatory function both in vitro and in vivo. Consequently, we have generated MSCs from human induced pluripotent stem (IPS-MSCs) cells that share similar properties with embryonic stem cells (ES-MSCs). Herein, we compared the immunoregulatory properties of ES/IPS-MSCs with those of BM-MSCs and showed, for the first time, that IPS-derived MSCs display remarkable inhibition of NK-cell proliferation and cytolytic function in a similar way to ES-MSCs. Both MSCs disrupt NK-cell cytolytic machinery in the same fashion that BM-MSCs, by down-regulating the expression of different activation markers and ERK1/2 signaling, leading to an impairment to form immunologic synapses with target cells and, therefore, secretion of cytotoxic granules. In addition, they are more resistant than adult BM-MSCs to preactivated

NK cells. IPS-MSCs could represent an attractive alternative source of immunoregulatory cells, and their capacity to impair NK-cell cytotoxicity constitutes a complex mechanism to prevent allograft rejection.

Glavaski-Joksimovic, A., et al. (2010). "Glial cell line-derived neurotrophic factor-secreting genetically modified human bone marrow-derived mesenchymal stem cells promote recovery in a rat model of Parkinson's disease." J Neurosci Res **88**(12): 2669-2681.

Parkinson's disease (PD) is a neurodegenerative disease characterized by progressive degeneration of nigrostriatal dopaminergic (DA) neurons. The therapeutic potential of glial cell line-derived neurotrophic factor (GDNF), the most potent neurotrophic factor for DA neurons, has been demonstrated in many experimental models of PD. However, chronic delivery of GDNF to DA neurons in the brain remains an unmet challenge. Here, we report the effects of GDNF-releasing Notch-induced human bone marrow-derived mesenchymal stem cells (MSC) grafted into striatum of the 6-hydroxydopamine (6-OHDA) progressively lesioned rat model of PD. Human MSC, obtained from bone marrow aspirates of young, healthy adult volunteers, were transiently transfected with the intracellular domain of the Notch1 gene (NICD) to generate SB623 cells. SB623 cells expressing GDNF and/or humanized Renilla green fluorescent protein (hrGFP) following lentiviral transduction or nontransduced cells were stereotaxically placed into rat striatum 1 week after a unilateral partial 6-OHDA striatal lesion. At 4 weeks, rats that had received GDNF-transduced SB623 cells had significantly decreased amphetamine-induced rotation compared with control rats, although this effect was not observed in rats that received GFP-transduced or nontransduced SB623 cells. At 5 weeks, rejuvenated tyrosine hydroxylase-immunoreactive (TH-IR) fibers that appeared to be host DA axons were observed in and around grafts. This effect was more prominent in rats that received GDNF-secreting cells and was not observed in controls. These observations suggest that human bone-marrow derived MSC, genetically modified to secrete GDNF, hold potential as an allogeneic or autologous stem cell therapy for PD.

Golpanian, S., et al. (2016). "Rationale and design of the allogeneiC human mesenchymal stem cells (hMSC) in patients with aging fRAilTy via intravenoUS delivery (CRATUS) study: A phase I/II, randomized, blinded and placebo controlled trial to evaluate the safety and potential efficacy of allogeneic human mesenchymal stem cell infusion in patients with aging frailty." Oncotarget **7**(11): 11899-11912.

Frailty is a syndrome associated with reduced physiological reserves that increases an individual's vulnerability for developing increased morbidity and/or mortality. While most clinical trials have focused on exercise, nutrition, pharmacologic agents, or a multifactorial approach for the prevention and attenuation of frailty, none have studied the use of cell-based therapies. We hypothesize that the application of allogeneic human mesenchymal stem cells (allo-hMSCs) as a therapeutic agent for individuals with frailty is safe and efficacious. The CRATUS trial comprises an initial non-blinded phase I study, followed by a blinded, randomized phase I/II study (with an optional follow-up phase) that will address the safety and pre-specified beneficial effects in patients with the aging frailty syndrome. In the initial phase I protocol, allo-hMSCs will be administered in escalating doses via peripheral intravenous infusion (n=15) to patients allocated to three treatment groups: Group 1 (n=5, 20 million allo-hMSCs), Group 2 (n=5, 100 million allo-hMSCs), and Group 3 (n=5, 200 million allo-hMSCs). Subsequently, in the randomized phase, allo-hMSCs or matched placebo will be administered to patients (n=30) randomly allocated in a 1:1:1 ratio to one of two doses of MSCs versus placebo: Group A (n=10, 100 million allo-hMSCs), Group B (n=10, 200 million allo-hMSCs), and Group C (n=10, placebo). Primary and secondary objectives are, respectively, to demonstrate the safety and efficacy of allo-hMSCs administered in frail older individuals. This study will determine the safety of intravenous infusion of stem cells and compare phenotypic outcomes in patients with aging frailty.

Goncalves, M. A., et al. (2006). "Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion." Hum Mol Genet **15**(2): 213-221.

Duchenne muscular dystrophy (DMD) is the most prevalent inheritable muscle disease. It is caused by mutations in the approximately 2.5-megabase dystrophin (Dys) encoding gene. Therapeutic attempts at DMD have relied on injection of allogeneic Dys-positive myoblasts. The immune rejection of these cells and their limited availability have prompted the search for alternative therapies and sources of myogenic cells. Stem cell-based gene therapy aims to restore tissue function by the transplantation of gene-corrected autologous cells. It depends on (i) the capacity of stem cells to participate in tissue regeneration and (ii) the efficient genetic correction of defective autologous stem cells. We explored the potential of bone marrow-derived human mesenchymal stem cells (hMSCs) genetically modified with the full-length Dys-coding sequence to engage in myogenesis. By tagging hMSCs

with enhanced green fluorescent protein (EGFP) or the membrane dye PKH26, we demonstrated that they could participate in myotube formation when cultured together with differentiating human myoblasts. Experiments performed with EGFP-marked hMSCs and DsRed-labeled DMD myoblasts revealed that the EGFP-positive DMD myotubes were also DsRed-positive indicating that hMSCs participate in human myogenesis through cellular fusion. Finally, we showed that hMSCs transduced with a tropism-modified high-capacity hybrid viral vector encoding full-length Dys could complement the genetic defect of DMD myotubes.

Goncalves Ndo, N., et al. (2016). "Effect of Melatonin in Epithelial Mesenchymal Transition Markers and Invasive Properties of Breast Cancer Stem Cells of Canine and Human Cell Lines." PLoS One **11**(3): e0150407.

Cancer stem cells (CSCs) have been associated with metastasis and therapeutic resistance and can be generated via epithelial mesenchymal transition (EMT). Some studies suggest that the hormone melatonin acts in CSCs and may participate in the inhibition of the EMT. The objectives of this study were to evaluate the formation of mammospheres from the canine and human breast cancer cell lines, CMT-U229 and MCF-7, and the effects of melatonin treatment on the modulation of stem cell and EMT molecular markers: OCT4, E-cadherin, N-cadherin and vimentin, as well as on cell viability and invasiveness of the cells from mammospheres. The CMT-U229 and MCF-7 cell lines were subjected to three-dimensional culture in special medium for stem cells. The phenotype of mammospheres was first evaluated by flow cytometry (CD44(+)/CD24(low/-) marking). Cell viability was measured by MTT colorimetric assay and the expression of the proteins OCT4, E-cadherin, N-cadherin and vimentin was evaluated by immunofluorescence and quantified by optical densitometry. The analysis of cell migration and invasion was performed in Boyden Chamber. Flow cytometry proved the stem cell phenotype with CD44(+)/CD24(low/-) positive marking for both cell lines. Cell viability of CMT-U229 and MCF-7 cells was reduced after treatment with 1mM melatonin for 24 h ($P<0.05$). Immunofluorescence staining showed increased E-cadherin expression ($P<0.05$) and decreased expression of OCT4, N-cadherin and vimentin ($P<0.05$) in both cell lines after treatment with 1 mM melatonin for 24 hours. Moreover, treatment with melatonin was able to reduce cell migration and invasion in both cell lines when compared to control group ($P<0.05$). Our results demonstrate that melatonin shows an inhibitory role in the viability and invasiveness of breast cancer mammospheres as well as in modulating the expression of proteins related to EMT in breast CSCs, suggesting its potential anti-metastatic role in canine and human breast cancer cell lines.

Guerrero, J., et al. (2013). "Cell interactions between human progenitor-derived endothelial cells and human mesenchymal stem cells in a three-dimensional macroporous polysaccharide-based scaffold promote osteogenesis." Acta Biomater **9**(9): 8200-8213.

Several studies have reported the benefits of mesenchymal stem cells (MSCs) for bone tissue engineering. However, vascularization remains one of the main obstacles that must be overcome to reconstruct large bone defects. In vitro prevascularization of the three-dimensional (3-D) constructs using co-cultures of human progenitor-derived endothelial cells (PDECs) with human bone marrow mesenchymal stem cells (HBMSCs) appeared as a potential strategy. However, the crosstalk between the two lineages has been studied in two-dimensional (2-D), but remains unknown in 3-D. The aim of this study is to investigate the cell interactions between PDECs and HBMSCs in a porous matrix composed of polysaccharides. This biodegradable scaffold promotes cell interactions by inducing multicellular aggregates composed of HBMSCs surrounded by PDECs. Cell aggregation contributes to the formation of junctional proteins composed of Connexin43 (Cx43) and VE-cadherin, and an activation of osteoblastic differentiation of HBMSCs stimulated by the presence of PDECs. Inhibition of Cx43 by mimetic peptide 43GAP27 induced a decrease in mRNA levels of Cx43 and all the bone-specific markers. Finally, subcutaneous implantations for 3 and 8 weeks in NOG mice revealed an increase in osteoid formation with the tissue-engineered constructs seeded with HBMSCs/PDECs compared with those loaded with HBMSCs alone. Taking together, these results demonstrate that this 3-D microenvironment favored cell communication, osteogenesis and bone formation.

Haack-Sorensen, M., et al. (2008). "Comparison of different culture conditions for human mesenchymal stromal cells for clinical stem cell therapy." Scand J Clin Lab Invest **68**(3): 192-203.

OBJECTIVE: Mesenchymal stromal cells (MSCs) from adult bone marrow (BM) are considered potential candidates for therapeutic neovascularization in cardiovascular disease. When implementing results from animal trials in clinical treatment, it is essential to isolate and expand the MSCs under conditions following good manufacturing practice (GMP). The aims of the study were first to establish culture conditions following GMP quality demands for human MSC expansion and differentiation for use in clinical

trials, and second to compare these MSCs with MSCs derived from culture in four media commonly used for MSC cultivation in animal studies simulating clinical stem cell therapy. MATERIAL AND METHODS: Human mononuclear cells (MNCs) were isolated from BM aspirates by density gradient centrifugation and cultivated in a GMP-accepted medium (EMEA medium) or in one of four other media. RESULTS: FACS analysis showed that the plastic-adherent MSCs cultured in EMEA medium or in the other four media were identically negative for the haematopoietic surface markers CD45 and CD34 and positive for CD105, CD73, CD90, CD166 and CD13, which in combined expression is characteristic of MSCs. MSC stimulation with vascular endothelial growth factor (VEGF) increased expression of the characteristic endothelial genes KDR and von Willebrand factor; the von Willebrand factor and CD31 at protein level as well as the capacity to develop capillary-like structures. CONCLUSIONS: We established culture conditions with a GMP compliant medium for MSC cultivation, expansion and differentiation. The expanded and differentiated MSCs can be used in autologous mesenchymal stromal cell therapy in patients with ischaemic heart disease.

Hajizadeh-Saffar, E., et al. (2015). "Inducible VEGF expression by human embryonic stem cell-derived mesenchymal stromal cells reduces the minimal islet mass required to reverse diabetes." Sci Rep **5**: 9322.

UNLABELLED: Islet transplantation has been hampered by loss of function due to poor revascularization. We hypothesize that co-transplantation of islets with human embryonic stem cell-derived mesenchymal stromal cells that conditionally overexpress VEGF (hESC-MSC:VEGF) may augment islet revascularization and reduce the minimal islet mass required to reverse diabetes in mice. HESC-MSCs were transduced by recombinant lentiviruses that allowed conditional (Dox-regulated) overexpression of VEGF. HESC-MSC: VEGF were characterized by tube formation assay. After co-transplantation of hESC-MSC:VEGF with murine islets in collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, we measured blood glucose, body weight, glucose tolerance and serum C-peptide. As control, islets were transplanted alone or with non-transduced hESC-MSCs. Next, we compared functional parameters of 400 islets alone versus 200 islets co-transplanted with hESC-MSC:VEGF. As control, 200 islets were transplanted alone. Metabolic function of islets transplanted with hESC-MSC:VEGF significantly improved, accompanied by superior graft revascularization, compared with control groups. Transplantation of 200 islets with hESC-MSC:VEGF showed superior function over 400 islets alone. We conclude that co-transplantation of islets with VEGF-expressing hESC-MSCs allowed for at least a 50% reduction in minimal islet mass required to reverse diabetes in mice. This approach may contribute to alleviate the need for multiple donor organs per patient.

Hamouda, H., et al. (2013). "N-glycosylation profile of undifferentiated and adipogenically differentiated human bone marrow mesenchymal stem cells: towards a next generation of stem cell markers." Stem Cells Dev **22**(23): 3100-3113.

Mesenchymal stem cells (MSCs) are multipotent cells that are easy to isolate and expand, develop into several tissues, including fat, migrate to diseased organs, have immunosuppressive properties and secrete regenerative factors. This makes MSCs ideal for regenerative medicine. For application and regulatory purposes, knowledge of (bio)markers characterizing MSCs and their development stages is of paramount importance. The cell surface is coated with glycans that possess lineage-specific nature, which makes glycans to be promising candidate markers. In the context of soft tissue generation, we aimed to identify glycans that could be markers for MSCs and their adipogenically differentiated progeny. MSCs were isolated from human bone marrow, adipogenically stimulated for 15 days and adipogenesis was verified by staining the lipid droplets and quantitative real time polymerase chain reaction of the marker genes peroxisome proliferator-activated receptor gamma (PPARG) and fatty acid binding protein-4 (FABP4). Using matrix-assisted laser desorption-ionization-time of flight mass spectrometry combined with exoglycosidase digestions, we report for the first time the N-glycome of MSCs during adipogenic differentiation. We were able to detect more than 100 different N-glycans, including high-mannose, hybrid, and complex N-glycans, as well as poly-N-acetyllactosamine chains. Adipogenesis was accompanied by an increased amount of biantennary fucosylated structures, decreased amount of fucosylated, afucosylated tri- and tetraantennary structures and increased sialylation. N-glycans H6N5F1 and H7N6F1 were significantly overexpressed in undifferentiated MSCs while H3N4F1 and H5N4F3 were upregulated in adipogenically differentiated MSCs. These glycan structures are promising candidate markers to detect and distinguish MSCs and their adipogenic progeny.

Hayashi, N., et al. (2009). "Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells support hematopoietic recovery of X-irradiated human CD34+ cells." Life Sci **84**(17-18): 598-605.

AIMS: The potential of human mesenchymal stem cell-like stroma prepared from placental/umbilical

cord blood for hematopoietic regeneration by X-irradiated hematopoietic stem cells is herein assessed. MAIN METHODS: Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells were applied to a regenerative ex vivo expansion of X-irradiated human CD34+ cells in a serum-free liquid culture supplemented with a combination of interleukine-3 plus stem cell factor plus thrombopoietin. KEY FINDINGS: The total number of cells and of lineage-committed myeloid hematopoietic progenitor cells generated in the co-culture of both non-irradiated and X-irradiated cells with stromal cells was significantly higher than those in the stroma-free culture. In addition, the number of CD34+ cells and CD34+/CD38- cells, immature hematopoietic stem/progenitor cells also increased more than the stroma-free culture. The stromal cells produced various types of cytokines, although there was little difference between the co-cultures of non-irradiated and X-irradiated cells with stromal cells. Furthermore, when X-irradiated cells came in contact with stromal cells for 16 h before cytokine stimulation, a similar degree of hematopoiesis was observed, thus suggesting the critical role of cell-to-cell interaction. SIGNIFICANCE: The present results showed the potential efficacy of human mesenchymal stem cell-like stroma for hematopoietic regeneration from irradiated hematopoietic stem/progenitor cells.

Hayashi, N., et al. (2009). "Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells support hematopoietic recovery of X-irradiated human CD34+ cells." Life Sci **84**(17-18): 598-605.

AIMS: The potential of human mesenchymal stem cell-like stroma prepared from placental/umbilical cord blood for hematopoietic regeneration by X-irradiated hematopoietic stem cells is herein assessed. MAIN METHODS: Placental/umbilical cord blood-derived mesenchymal stem cell-like stromal cells were applied to a regenerative ex vivo expansion of X-irradiated human CD34(+) cells in a serum-free liquid culture supplemented with a combination of interleukine-3 plus stem cell factor plus thrombopoietin. KEY FINDINGS: The total number of cells and of lineage-committed myeloid hematopoietic progenitor cells generated in the co-culture of both non-irradiated and X-irradiated cells with stromal cells was significantly higher than those in the stroma-free culture. In addition, the number of CD34(+) cells and CD34(+)/CD38(-) cells, immature hematopoietic stem/progenitor cells also increased more than the stroma-free culture. The stromal cells produced various types of cytokines, although there was little difference between the co-cultures of non-irradiated and X-irradiated cells with stromal cells. Furthermore, when X-irradiated cells came in contact with stromal cells for 16 h before cytokine stimulation, a similar degree of hematopoiesis was observed, thus suggesting the critical role of cell-to-cell interaction. SIGNIFICANCE: The present results showed the potential efficacy of human mesenchymal stem cell-like stroma for hematopoietic regeneration from irradiated hematopoietic stem/progenitor cells.

He, W., et al. (2010). "[Effect of ginsenoside Rg1 on the microenvironment dependent differentiation of human bone marrow mesenchymal stem cell to vaso-endothelioid formative cells in vitro]." Zhongguo Zhong Xi Yi Jie He Za Zhi **30**(11): 1201-1205.

OBJECTIVE: To investigate the effect of ginsenoside Rg1 on the microenvironment dependent differentiation of human mesenchymal stem cells (hMSCs) to vaso-endothelioid cells (VECs) in vitro. METHODS: The in vitro differentiation of hMSCs to VECs were established adopting the in vivo environment simulated semi-permeable membrane separated non-contact co-culturing method. The mRNA expressions of endothelial markers, such as platelet endothelial adhesive factor-1 (CD31), vascular hemophillia factor (vWF) and vascular endothelial cadherin (VE-cadherin) were analyzed by RT-PCR; the protein expressions of CD31 and vascular endothelial adhesive factor-1 (VCAM1) were detected by fluorescence immunohistochemistry; structural identification for the endothelial characteristics of differentiated hMSCs were made under electron microscopy; and the percentage of CD31 expression in differentiated hMSCs was determined by flow cytometry to explore the effect of ginsenoside Rg1 on the differentiation. RESULTS: The bone marrow mesenchymal stem cells co-cultured with mature endothelial membrane showed a microenvironment dependent capacity for differentiating to endothelium, with the morphological changes revealed starting from the 2nd week, showing cell body contraction, polygonal-shaped change; and at the 3rd week, the markedly speedily cell proliferation with elliptic or slabstone-like change of cells. High levels of classic endothelial cell markers, such as mRNA expressions of CD31, vWF, VE-cadherin, and protein expressions of CD31 and VCAM1, were shown; the typical weibel-palade body of endothelial cell was found in the differentiated cells. Moreover, percentage of CD31 expression in the differentiated hMSCs was increased after Rg1 treatment dose-dependently. CONCLUSION: Under the microenvironment of co-culture, hMSCs could differentiate into cells presenting the characteristics of endothelial cell in aspects of the morphology and ultrastructure of cells, as well as the gene and protein expressions of cell markers; ginsenoside Rg1 can promote the microenvironment

dependent differentiation of hMSCs to VECs system in vitro.

Hematti, P. (2011). "Human embryonic stem cell-derived mesenchymal stromal cells." Transfusion **51 Suppl 4**: 138S-144S.

Mesenchymal stromal cells (MSCs) originally isolated from marrow have multipotent differentiation potential and favorable immunomodulatory and anti-inflammatory properties that make them very attractive for regenerative cellular therapy. Cells with similar phenotypic characteristics have now been derived from almost all fetal, neonatal, and adult tissues; furthermore, more recently similar cells have also been generated from human embryonic stem cells (ESCs). Generation of MSCs from human ESCs provides an opportunity to study the developmental biology of human mesenchymal lineage generation in vitro. Generation of bone and cartilage from human ESC-derived MSCs and their functional characterization, both in vitro and in vivo, is also an active area of investigation as ESCs could provide an unlimited source of MSCs for potential repair of bone and cartilage defects. MSCs from adult sources are being investigated in numerous Phase I-III clinical trials for a wide variety of indications, mainly based on their immunomodulatory properties. Our group and others have shown MSCs derived from human ESCs possess immunomodulatory properties similar to marrow-derived MSCs. Immunomodulatory properties of ESC-derived MSCs could prove to be highly valuable for their potential clinical applications in the future as derivatives of human ESCs have already entered clinical arena in the context of Phase I clinical trials.

Hendijani, F., et al. (2015). "Effect of human Wharton's jelly mesenchymal stem cell secretome on proliferation, apoptosis and drug resistance of lung cancer cells." Res Pharm Sci **10**(2): 134-142.

Multipotent mesenchymal stem cells (MSCs) are recently found to alter the tumor condition. However their exact role in tumor development is not yet fully unraveled. MSCs were established to perform many of their actions through paracrine effect. Thus investigation of MSC secretome interaction with tumor cells may provide important information for scientists who are attempting to apply stem cells in the treatment of the disease. In this study we investigated the effect of human Wharton's jelly derived MSC (WJ-MSCs) secretome on proliferation, apoptotic potential of A549 lung cancer cells, and their response to the chemotherapeutic agent doxorubicin. WJ-MSCs were isolated from human umbilical cord and then characterized according to the International Society for Cellular Therapy criteria and WJ-MSC secretome was collected. BrdU cell proliferation assay and Annexin V-PI staining were used for the evaluation of cytotoxic and proapoptotic effects of WJ-MSC secretome on A549 cells. WJ-MSC secretome neither induced proliferation of lung cancer cells nor affected the apoptotic potential of the tumor cells. We also studied the combinatorial effect of WJ-MSC secretome and the anticancer drug doxorubicinwhich showed no induction of drug resistance when A549 cells was treated with combination of WJ-MSC secretome and doxorubicin. Although MSCs did not show antitumor properties, our in vitro results showed that MSC secretome was not tumorigenic and also did not make lung cancer cells resistant to doxorubicin. Thus MSC secretome could be considered safe for other medical purposes such as cardiovascular, neurodegenerative, and autoimmune diseases which may exist or occur in cancer patients.

Henrich, D., et al. (2013). "Human endothelial-like differentiated precursor cells maintain their endothelial characteristics when cocultured with mesenchymal stem cell and seeded onto human cancellous bone." Mediators Inflamm **2013**: 364591.

INTRODUCTION: Cancellous bone is frequently used for filling bone defects in a clinical setting. It provides favourable conditions for regenerative cells such as MSC and early EPC. The combination of MSC and EPC results in superior bone healing in experimental bone healing models. MATERIALS AND METHODS: We investigated the influence of osteogenic culture conditions on the endothelial properties of early EPC and the osteogenic properties of MSC when cocultured on cancellous bone. Additionally, cell adhesion, metabolic activity, and differentiation were assessed 2, 6, and 10 days after seeding. RESULTS: The number of adhering EPC and MSC decreased over time; however the cells remained metabolically active over the 10-day measurement period. In spite of a decline of lineage specific markers, cells maintained their differentiation to a reduced level. Osteogenic stimulation of EPC caused a decline but not abolishment of endothelial characteristics and did not induce osteogenic gene expression. Osteogenic stimulation of MSC significantly increased their metabolic activity whereas collagen-1alpha and alkaline phosphatase gene expressions declined. When cocultured with EPC, MSC's collagen-1alpha gene expression increased significantly. CONCLUSION: EPC and MSC can be cocultured in vitro on cancellous bone under osteogenic conditions, and coculturing EPC with MSC stabilizes the latter's collagen-1alpha gene expression.

Hill, A. J., et al. (2009). "Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after

intravitreal grafting in neonatal rats." Stem Cells Dev **18**(3): 399-409.

This study investigated the ability of mesenchymal stem cells (MSCs) derived from full-term human umbilical cord blood to survive, integrate and differentiate after intravitreal grafting to the degenerating neonatal rat retina following intracranial optic tract lesion. MSCs survived for 1 week in the absence of immunosuppression. When host animals were treated with cyclosporin A and dexamethasone to suppress inflammatory and immune responses, donor cells survived for at least 3 weeks, and were able to spread and cover the entire vitreal surface of the host retina. However, MSCs did not significantly integrate into or migrate through the retina. They also maintained their human antigenicity, and no indication of neural differentiation was observed in retinas where retinal ganglion cells either underwent severe degeneration or were lost. These results have provided the first in vivo evidence that MSCs derived from human umbilical cord blood can survive for a significant period of time when the host rat response is suppressed even for a short period. These results, together with the observation of a lack of neuronal differentiation and integration of MSCs after intravitreal grafting, has raised an important question as to the potential use of MSCs for neural repair through the replacement of lost neurons in the mammalian retina and central nervous system.

Imamura, H., et al. (2018). "An engineered cell sheet composed of human islets and human fibroblast, bone marrow-derived mesenchymal stem cells, or adipose-derived mesenchymal stem cells: An in vitro comparison study." Islets **10**(3): e1445948.

BACKGROUND: We previously reported the utility of engineered cell sheets composed of human islets and supporting cells in vitro and in vivo. It is unclear which type of supporting cell is most suitable for constructing cell sheets with human islets. The present study aimed to compare human fibroblasts, bone marrow-derived mesenchymal stem cells (BM-MSCs), and adipose-derived mesenchymal stem cells (ADSCs) as a supporting source for cell sheets. METHODS: Engineered cell sheets were fabricated with human islets using human fibroblasts, BM-MSCs, or ADSCs as supporting cells. The islet viability, recovery rate, glucose-stimulated insulin release (determined by the stimulation index), and cytokine secretion (TGF-beta1, IL-6, and VEGF) of groups-including an islet-alone group as a control-were compared. RESULTS: All three sheet groups consistently exhibited higher viability, recovery rate, and stimulation index values than the islet-alone group. The ADSC group showed the highest viability and recovery rate among the three sheet groups. There were no discernible differences in the stimulation index values of the groups. The fibroblast group exhibited significantly higher TGF-beta1 values in comparison to the other groups. The IL-6 level of the ADSC group was more than five times higher than that of the other groups. The ADSC group showed the VEGF level; however, it did not differ from that of the BM-MSC group to a statistically significant extent. CONCLUSION: Engineered cell sheets composed of islets and supporting cells had a cytoprotective effect on islets. These results suggest that individual cell types could be a more attractive source for crafting engineered cell sheets in comparison to islets alone.

Ishii, T., et al. (2010). "In vitro hepatic maturation of human embryonic stem cells by using a mesenchymal cell line derived from murine fetal livers." Cell Tissue Res **339**(3): 505-512.

Hepatocytes derived from human embryonic stem cells (hESCs) are an attractive cell source for regenerative medicine. We previously reported the differentiation of hESCs into alpha-fetoprotein (AFP)-producing endodermal cells by using extracellular matrix and growth factors. We also reported the establishment of the MLSgt20 cell line, which was derived from mesenchymal cells residing in murine fetal livers and accelerated the hepatic maturation of both murine hepatic progenitor cells and murine ESCs. In this study, hESC-derived AFP-producing cells were isolated by using a flow cytometer and co-cultured with MLSgt20 cells. The co-cultured hESC-derived AFP-producing cells had the immunocytological characteristics of hepatocytes, expressed mature hepatocyte markers (as indicated by reverse transcription and the polymerase chain reaction), and displayed higher hepatocyte functions including ammonia removal, cytochrome P450 3A4/7 activity, and the ability to produce and store glycogen. However, the MLSgt20 cells did not directly cause undifferentiated hESCs to mature into hepatocyte-like cells. The co-culture method was thus successfully shown to induce the differentiation of hESC-derived endodermal cells into functional hepatocyte-like cells.

Ishimine, H., et al. (2013). "N-Cadherin is a prospective cell surface marker of human mesenchymal stem cells that have high ability for cardiomyocyte differentiation." Biochem Biophys Res Commun **438**(4): 753-759.

Mesenchymal stem cells (MSCs) are among the most promising sources of stem cells for regenerative medicine. However, the range of their differentiation ability is very limited. In this study, we explored prospective cell surface markers of human MSCs that readily differentiate into cardiomyocytes. When the cardiomyogenic differentiation potential and

the expression of cell surface markers involved in heart development were analyzed using various immortalized human MSC lines, the MSCs with high expression of N-cadherin showed a higher probability of differentiation into beating cardiomyocytes. The differentiated cardiomyocytes expressed terminally differentiated cardiomyocyte-specific markers such as alpha-actinin, cardiac troponin T, and connexin-43. A similar correlation was observed with primary human MSCs derived from bone marrow and adipose tissue. Moreover, N-cadherin-positive MSCs isolated with N-cadherin antibody-conjugated magnetic beads showed an apparently higher ability to differentiate into cardiomyocytes than the N-cadherin-negative population. Quantitative polymerase chain reaction analyses demonstrated that the N-cadherin-positive population expressed significantly elevated levels of cardiomyogenic progenitor-specific transcription factors, including Nkx2.5, Hand1, and GATA4 mRNAs. Our results suggest that N-cadherin is a novel prospective cell surface marker of human MSCs that show a better ability for cardiomyocyte differentiation.

Itokazu, M., et al. (2016). "Transplantation of Scaffold-Free Cartilage-Like Cell-Sheets Made from Human Bone Marrow Mesenchymal Stem Cells for Cartilage Repair: A Preclinical Study." Cartilage **7**(4): 361-372.

OBJECTIVE: The object of this study was to determine culture conditions that create stable scaffold-free cartilage-like cell-sheets from human bone marrow-derived mesenchymal stem cells (hBMSCs) and to assess their effects after transplantation into osteochondral defects in nude rats. DESIGN: (Experiment 1) The hBMSCs were harvested from 3 males, the proliferative and chondrogenic capacities were assessed at passage 1, and the cells were expanded in 3 different culture conditions: (1) 5% fetal bovine serum (FBS), (2) 10% FBS, and (3) 5% FBS with fibroblast growth factor 2 (FGF-2). The cells were harvested and made chondrogenic pellet culture. The cell proliferation rate, glycosaminoglycan/DNA ratio, and safranin-O staining intensity of pellets cultured condition 3 were higher than those of conditions 1 and 2. (Experiment 2) The hBMSCs were expanded and passaged 3 times under culture condition 3, and fabricate the cell-sheets in chondrogenic medium either with or without FBS. The cell-sheets fabricated with FBS maintained their size with flat edges. (Experiment 3) The cell-sheets were transplanted into osteochondral defects in nude rats. Histological analysis was performed at 2, 4, and 12 weeks after surgery. RESULTS: The osteochondral repair was better after sheet transplantation than in the control group and significantly improved Wakitani score. Immunostaining with human-specific vimentin antibody showed that the transplanted cells became fewer and disappeared at 12 weeks. CONCLUSIONS: These results indicate that culture with FGF-2 may help to quickly generate sufficient numbers of cells to create stable and reliable scaffold-free cartilage-like cell-sheets, which contribute to the regeneration of osteochondral defects.

Jarvinen, L., et al. (2008). "Lung resident mesenchymal stem cells isolated from human lung allografts inhibit T cell proliferation via a soluble mediator." J Immunol **181**(6): 4389-4396.

Development of allograft rejection continues to be the major determinant of morbidity and mortality postlung transplantation. We have recently demonstrated that a population of donor-derived mesenchymal stem cells is present in human lung allografts and can be isolated and expanded ex vivo. In this study, we investigated the impact of lung resident mesenchymal stem cells (LR-MSCs), derived from allografts of human lung transplant recipients, on T cell activation in vitro. Similar to bone marrow-derived MSCs, LR-MSCs did not express MHC II or the costimulatory molecules CD80 or CD86. In vitro, LR-MSCs profoundly suppressed the proliferative capacity of T cells in response to a mitogenic or an allogeneic stimulus. The immunosuppressive function of LR-MSCs was also noted in the absence of direct cell contact, indicating that LR-MSCs mediated their effect predominantly via a soluble mediator. LR-MSCs isolated from lung transplant recipients demonstrated PGE(2) secretion at baseline (385 +/- 375 pg/ml), which increased in response to IL-1beta (1149 +/- 1081 pg/ml). The addition of PG synthesis inhibitors (indomethacin and NS-398) substantially abrogated LR-MSC-mediated immunosuppression, indicating that PGE(2) may be one of the major soluble mediators impacting T cell activity. This is the first report to demonstrate that human tissue-derived MSCs isolated from an allogeneic environment have the potential to mediate immunological responses in vitro.

Jeon, E. S., et al. (2010). "Ovarian cancer-derived lysophosphatidic acid stimulates secretion of VEGF and stromal cell-derived factor-1 alpha from human mesenchymal stem cells." Exp Mol Med **42**(4): 280-293.

Lysophosphatidic acid (LPA) stimulates growth and invasion of ovarian cancer cells and tumor angiogenesis. Cancer-derived LPA induces differentiation of human adipose tissue-derived mesenchymal stem cells (hASCs) to alpha-smooth muscle actin (alpha-SMA)-positive cancer-associated fibroblasts. Presently, we explored whether cancer-derived LPA regulates secretion of pro-angiogenic factors from hASCs. Conditioned medium (CM) from the OVCAR-3 and SKOV3 ovarian cancer cell lines

stimulated secretion angiogenic factors such as stromal-derived factor-1 alpha (SDF-1 alpha) and VEGF from hASCs. Pretreatment with the LPA receptor inhibitor Ki16425 or short hairpin RNA lentiviral silencing of the LPA((1)) receptor abrogated the cancer CM-stimulated expression of alpha-SMA, SDF-1, and VEGF from hASCs. LPA induced expression of myocardin and myocardin-related transcription factor-A, transcription factors involved in smooth muscle differentiation, in hASCs. siRNA-mediated depletion of endogenous myocardin and MRTF-A abrogated the expression of alpha-SMA, but not SDF-1 and VEGF. LPA activated RhoA in hASCs and pretreatment with the Rho kinase inhibitor Y27632 completely abrogated the LPA-induced expression of alpha-SMA, SDF-1, and VEGF in hASCs. Moreover, LPA-induced alpha-SMA expression was abrogated by treatment with the ERK inhibitor U0126 or the phosphoinositide-3-kinase inhibitor LY294002, but not the PLC inhibitor U73122. LPA-induced VEGF secretion was inhibited by LY294002, whereas LPA-induced SDF-1 secretion was markedly attenuated by U0126, U73122, and LY294002. These results suggest that cancer-secreted LPA induces differentiation of hASCs to cancer-associated fibroblasts through multiple signaling pathways involving Rho kinase, ERK, PLC, and phosphoinositide-3-kinase.

Jeon, E. S., et al. (2005). "Role of MEK-ERK pathway in sphingosylphosphorylcholine-induced cell death in human adipose tissue-derived mesenchymal stem cells." Biochim Biophys Acta **1734**(1): 25-33.

Sphingosylphosphorylcholine (SPC) is a bioactive lipid molecule involved in a variety of cellular responses. In the present study, we demonstrated that treatment of human adipose tissue-derived mesenchymal stem cells (hATSCs) with D-erythro-SPC resulted in apoptosis-like cell death, as demonstrated by decreased cell viability, DNA strand breaks, the increase of sub-G1 fraction, cytochrome c release into cytosol, and activation of caspase-3. In contrast, the exposure of hATSCs to L-threo-SPC did not induce the cell death, suggesting that the SPC-induced cell death was selective for the D-erythro-stereoisomer of SPC. The D-erythro-SPC-induced cell death was prevented by DEVD-CHO, a caspase-3 specific inhibitor, and Z-VAD-FMK, a general caspase inhibitor, suggesting that the SPC-induced cell death of hATSCs occurs through the cytochrome c- and caspase-3-dependent pathways. In addition, D-erythro-SPC treatment stimulated the activation of mitogen-activated protein kinases, such as ERK and c-Jun NH2-terminal protein kinase (JNK), and the D-erythro-SPC-induced cell death was completely prevented by pretreatment with the MEK inhibitor, U0126, but not by pretreatment with the JNK inhibitor, SP600125, and the p38 MAPK inhibitor, SB202190, suggesting a specific involvement of ERK in the D-erythro-SPC-induced cell death. Pretreatment with U0126 attenuated the D-erythro-SPC-induced release of cytochrome c. From these results, we suggest that ERK is involved in the SPC-induced cell death of hATSC through stimulation of the cytochrome c/caspase-3-dependent pathway.

Jeon, Y. J., et al. (2016). "Comparative Analysis of Human Mesenchymal Stem Cells Derived From Bone Marrow, Placenta, and Adipose Tissue as Sources of Cell Therapy." J Cell Biochem **117**(5): 1112-1125.

Various source-derived mesenchymal stem cells (MSCs) with multipotent capabilities were considered for cell therapeutics of incurable diseases. The applicability of MSCs depends on the cellular source and on their different in vivo functions, despite having similar phenotypic and cytological characteristics. We characterized MSCs from different sources, including human bone marrow (BM), placenta (PL), and adipose tissue (AT), in terms of the phenotype, surface antigen expression, differentiation ability, proteome reference map, and blood flow recovery in a hindlimb ischemic disease model. The MSCs exhibit different differentiation potentials depending on the cellular source despite having similar phenotypic and surface antigen expression. We identified approximately 90 differentially regulated proteins. Most up- or down-regulated proteins show cytoskeletal or oxidative stress, peroxiredoxin, and apoptosis roles according to their functional involvement. In addition, the PL-MSCs retained a higher therapeutic efficacy than the BM- and AT-MSCs in the hindlimb ischemic disease model. In summary, we examined differentially expressed key regulatory factors for MSCs that were obtained from several cellular sources and demonstrated their differentially expressed proteome profiles. Our results indicate that primitive PL-MSCs have biological advantages relative to those from other sources, making PL-MSCs a useful model for clinical applications of cell therapy.

Jezierska-Wozniak, K., et al. (2018). "Migration of human mesenchymal stem cells stimulated with pulsed electric field and the dynamics of the cell surface glycosylation." Adv Clin Exp Med **27**(9): 1181-1193.

BACKGROUND: The analysis of the stem cells' glycome dynamics at different stages of differentiation and migration makes possible the exploration of the cell surface glycans as markers of the stem cell functional status, and, in the future, compatibility between transplanted cell and host environment. OBJECTIVES: The objective of our study was to develop novel techniques of investigating

cell motility and to assess whether the electric field of the therapeutic spinal cord stimulation system used in vivo contributes to the migration of human mesenchymal stem cells (hMSCs) in vitro. MATERIAL AND METHODS: We have investigated the electrotaxis of bone marrow-derived MSCs using pulsed electric field (PEF) in the range of 16-80 mV/mm and the frequency of 130 Hz and 240 Hz. The PEF-related dynamics of the cell surface glycosylation was evaluated using 6 plant lectins recognizing individual glycans. RESULTS: Pulsed electric field at physiological levels (10 mV/mm; 130 Hz) did not influence cellular motility in vitro, which may correspond to the maintenance of the transplanted cells at the lesion site in vivo. An increase of the PEF intensity and the frequency exceeding physiological levels resulted in an increase in the cellular migration rate in vitro. Pulsed electric field elevated above physiological intensity and frequency (40-80 mV/mm; 240 Hz), but not at physiological levels, resulted in changes of the cell surface glycosylation. CONCLUSIONS: We found the described approach convenient for investigations and for the in vitro modeling of the cellular systems intended for the regenerative cell transplantations in vivo. Probing cell surface glycomes may provide valuable biomarkers to assess the competence of transplanted cells.

Joddar, B., et al. (2018). "A Contact-Based Method for Differentiation of Human Mesenchymal Stem Cells into an Endothelial Cell-Phenotype." Cell Biochem Biophys **76**(1-2): 187-195.

Adult stem cells such as mesenchymal stem cells (MSC) are known to possess the ability to augment neovascularization processes and are thus widely popular as an autologous source of progenitor cells. However there is a huge gap in our current knowledge of mechanisms involved in differentiating MSC into endothelial cells (EC), essential for lining engineered blood vessels. To fill up this gap, we attempted to differentiate human MSC into EC, by culturing the former onto chemically fixed layers of EC or its ECM, respectively. We expected direct contact of MSC when cultured atop fixed EC or its ECM, would coax the former to differentiate into EC. Results showed that human MSC cultured atop chemically fixed EC or its ECM using EC-medium showed enhanced expression of CD31, a marker for EC, compared to other cases. Further in all human MSC cultured using EC-medium, typically characteristic cobble stone shaped morphologies were noted in comparison to cells cultured using MSC medium, implying that the differentiated cells were sensitive to soluble VEGF supplementation present in the EC-medium. Results will enhance and affect therapies utilizing autologous MSC as a cell source for generating vascular cells to be used in a variety of tissue engineering applications.

Joensuu, K., et al. (2011). "Interaction between marrow-derived human mesenchymal stem cells and peripheral blood mononuclear cells in endothelial cell differentiation." Scand J Surg **100**(3): 216-222.

BACKGROUND AND AIMS: In adult connective tissues, mesenchymal stem cells (MSCs) play a key role in normal tissue turnover and repair. MSCs can participate in these processes not only through proliferation and differentiation but also through paracrine/autocrine functions. These characteristics make MSCs the optimal target in the development of cell-based therapies. This study describes a novel interaction between human MSC and blood mononuclear cells (MNCs), resulting in formation of blood vessel-like structures. MATERIALS AND METHODS: Human marrow-derived MSCs and peripheral blood MNCs were co-cultured in monolayer cultures as well as in bovine collagen sponge up to 20 days. No exogenously supplied growth factors were applied. Morphological changes and formations of three dimensional structures were detected by light microscopy. The process was further stu-died for the expression of different endothelial cell markers. The expression of PECAM-1 and endoglin was studied by immunohistochemistry and the expression of vascular endothelial growth factor receptors 1 and 2 using quantitative real time PCR. RESULTS: In co-cultures of human MSCs and MNCs, the previously nonadherent cells attached and started to elongate and formed tube-like structures within one week. At day 10, elongated PECAM-1 and endoglin expressing cells were detected in co-cultures. At day 20, PECAM-1 and endoglin-positive vessel-like structures were observed. VEGFR1 was up-regulated in co-cultures after 10 days, and expression levels increased with time. No PECAM-1, endoglin or VEGFR1 expressing cells were discovered in MSC-cultures without MNCs at any time point. CONCLUSIONS: This study demonstrates induction of endothelial differentiation in co-cultures of human MSCs and MNCs, indicating a mechanism by which local application of MSCs could induce angiogenesis in vivo.

Ju, G. Q., et al. (2015). "Microvesicles derived from human umbilical cord mesenchymal stem cells facilitate tubular epithelial cell dedifferentiation and growth via hepatocyte growth factor induction." PLoS One **10**(3): e0121534.

During acute kidney injury (AKI), tubular cell dedifferentiation initiates cell regeneration; hepatocyte growth factor (HGF) is involved in modulating cell dedifferentiation. Mesenchymal stem cell (MSC)-

derived microvesicles (MVs) deliver RNA into injured tubular cells and alter their gene expression, thus regenerating these cells. We boldly speculated that MVs might induce HGF synthesis via RNA transfer, thereby facilitating tubular cell dedifferentiation and regeneration. In a rat model of unilateral AKI, the administration of MVs promoted kidney recovery. One of the mechanisms of action is the acceleration of tubular cell dedifferentiation and growth. Both in vivo and in vitro, rat HGF expression in damaged rat tubular cells was greatly enhanced by MV treatment. In addition, human HGF mRNA present in MVs was delivered into rat tubular cells and translated into the HGF protein as another mechanism of HGF induction. RNase treatment abrogated all MV effects. In the in vitro experimental setting, the conditioned medium of MV-treated injured tubular cells, which contains a higher concentration of HGF, strongly stimulated cell dedifferentiation and growth, as well as Erk1/2 signaling activation. Intriguingly, these effects were completely abrogated by either c-Met inhibitor or MEK inhibitor, suggesting that HGF induction is a crucial contributor to the acceleration of cell dedifferentiation and growth. All these findings indicate that MV-induced HGF synthesis in damaged tubular cells via RNA transfer facilitates cell dedifferentiation and growth, which are important regenerative mechanisms.

Kadam, S., et al. (2010). "Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes." Rev Diabet Stud **7**(2): 168-182.

Placental tissue holds great promise as a source of cells for regenerative medicine due to its plasticity, and easy availability. Human placenta-derived mesenchymal stem cells (hPDMSCs) have the potential to differentiate into insulin-producing cells. Upon transplantation, they can reverse experimental diabetes in mice. However, it is not known whether culture-expanded undifferentiated hPDMSCs are capable of restoring normoglycemia upon transplantation in streptozotocin (STZ)-induced diabetic mice. Hence we prepared long-term cultures of hPDMSCs from the chorionic villi of full-term human placenta. Flow cytometry analyses and immunocytochemistry study revealed bonafide mesenchymal nature of the isolated hPDMSCs. These cultures could differentiate into adipogenic, oesteogenic, chondrogenic, and neuronal lineages on exposure to lineage-specific cocktails. Furthermore, we showed that hPDMSCs can form islet-like cell clusters (ILCs) on stepwise exposure to serum-free defined media containing specific growth factors and differentiating agents. qRT-PCR showed the expression of insulin, glucagon, and somatostatin in undifferentiated hPDMSCs and in ILCs. Differentiated ILCs were found to express human insulin, glucagon, and somatostatin by immunocytochemistry. Additionally, ILCs also showed abundance of pancreatic transcription factors ngn3 and isl1. Both undifferentiated hPDMSCs and ILCs exihibited insulin secretion in response to glucose. Transplantation of hPDMSCs or ILCs derived from hPDMSCs in STZ-induced diabetic mice led to restoration of normoglycemia. Our results demonstrate, for the first time, reversal of hyperglycemia by undifferentiated hPDMSCs and ILCs derived from hPDMSCs. These results suggest human placenta-derived MSCs as an alternative source for cell replacement therapy in diabetes.

Kanemura, Y. (2010). "Development of cell-processing systems for human stem cells (neural stem cells, mesenchymal stem cells, and iPS cells) for regenerative medicine." Keio J Med **59**(2): 35-45.

Regenerative medicine using human stem cells is one of the newest and most promising fields for treating various intractable diseases and damaged organs. For clinical applications, choosing which human stem cells to use, i.e. according to tissue of origin and progenitor type, is a critical issue. Neural stem/progenitor cells (NSPCs) hold promise for treating various neurological diseases. We have shown that the transporter protein ABCB1 is predominantly expressed in immature human fetal NSPCs, and thus could be used as a phenotypic marker to investigate and monitor NSPCs in culture. We describe our proposed model for the in vitro proliferative process of aggregated human NSPCs and show that neurosphere enlargement and NSPC proliferation are mutually reinforcing. We have established that human neurospheres contain a heterogeneous cell population, knowledge that will contribute to the development of human neurospheres with desirable characteristics for clinical applications. Furthermore, decidua-derived mesenchymal cells (DMCs), which we isolated from human placenta, have unique properties as mesenchymal stem cells. They also generate a pericellular matrix (PCM-DM) that supports the growth and pluripotency of human embryonic stem cells and induced pluripotent stem cells (hiPS) cells. The newly developed re-programming techniques for generating hiPS cells should greatly contribute to cell therapies using human pluripotent stem cells, including those derived from DMCs. Our DMC-derived hiPS cells are a promising candidate source of allogeneic hiPS cells for clinical applications. We hope our findings will contribute to the development of cell-culture systems for generating human allogeneic stem cells for clinical use in regenerative medicine.

Kanzawa, M., et al. (2013). "WNT5A is a key regulator of the epithelial-mesenchymal transition and cancer stem cell properties in human gastric carcinoma cells." Pathobiology **80**(5): 235-244.

OBJECTIVE: Direct interaction with cancer-associated fibroblasts triggers WNT5A expression in human gastric carcinoma (GC) cells. In this study, we performed gene transduction experiments to investigate the significance of WNT5A in the GC tumor microenvironment. METHODS: Gene transduction (pWNT5A and shWNT5A) was performed in human GC-derived MKN-7 cells. Altered gene expression was examined by RT-PCR and cDNA microarray analysis. Immunohistochemical examination was carried out in human GC tissues. RESULTS: Transduction of exogenous WNT5A expression into MKN-7 cells upregulated genes related to the epithelial-mesenchymal transition (EMT) and cancer stem cells (CSCs), and the pWNT5A transfectant showed high tumorigenicity in vivo. These results were confirmed by knockdown experiments using a lentivirus expressing shWNT5A. A cDNA microarray analysis suggested that depletion of endogenous WNT5A downregulated genes involved in intracellular signaling, chemokine-cytokine interaction and focal adhesion. High levels of WNT5A expression were observed in 66% of GC cases, with significant correlation with histological type. Interestingly, in intestinal-type GCs, WNT5A expression was detected in the periphery of tumor nests. CONCLUSIONS: WNT5A regulates the induction of EMT and the maintenance of CSC properties in MKN-7 cells. WNT5A may play an important role in constructing an advantageous tumor microenvironment for the progression and development of human GC.

Karaoz, E., et al. (2011). "Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells." Histochem Cell Biol **136**(4): 455-473.

Dental pulp stem cells (hDP-SCs) were primarily derived from pulp tissues of primary incisors, exfoliated deciduous and permanent third molar teeth. To understand the characteristics of hDP-SCs from impacted third molar, proliferation capacities, gene expression profiles, phenotypic, ultrastructural, and differentiation characteristics were analyzed in comparison with human bone marrow-derived mesenchymal stem cells (hBM-MSCs), extensively. hDP-SCs showed more developed and metabolically active cells. Contrary to hBM-MSCs, hDP-SCs strongly expressed both cytokeratin (CK)-18 and -19, which could involve in odontoblast differentiation and dentine repair. The intrinsic neuro-glia characteristics of hDP-MSCs were demonstrated by the expression of several specific transcripts and proteins of neural stem cell and neurons. These cells not only differentiate into adipogenic, osteogenic, and chondrogenic lineage, but also share some special characteristics of expressing some neural stem cell and epithelial markers. Under defined conditions, hDP-SCs are able to differentiate into both neural and vascular endothelial cells in vitro. Dental pulp might provide an alternative source for human MSCs. hDP-SCs with a promising differentiation capacity could be easily isolated, and possible clinical use could be developed for neurodegenerative and oral diseases in the future.

Kasten, P., et al. (2008). "Instant stem cell therapy: characterization and concentration of human mesenchymal stem cells in vitro." Eur Cell Mater **16**: 47-55.

In regenerative medicine, there is an approach to avoid expansion of the mesenchymal stem cell (MSC) before implantation. The aim of this study was to compare methods for instant MSC therapy by use of a portable, automatic and closed system centrifuge that allows for the concentration of MSCs. The main outcome measures were the amount of MSCs per millilitre of bone marrow (BM), clusters of differentiation (CD), proliferation and differentiation capacities of the MSC. A volume reduction protocol was compared to the traditional laboratory methods of isolation using a Ficoll gradient and native BM. Fifty millilitres of BM were obtained from haematologically healthy male Caucasians (n=10, age 8 to 49 years). The number of colony forming units-fibroblast (CFU-F)/ml BM was highest in the centrifuge volume reduction protocol, followed by the native BM (not significant), the centrifuge Ficoll (p=0.042) and the manual Ficoll procedure (p=0.001). The MSC of all groups could differentiate into the mesenchymal lineages without significant differences between the groups. The CD pattern was identical for all groups: CD13+; CD 44+; CD73 +; CD90+; CD105+; HLA-A,B,C+; CD14-; CD34-; CD45-; CD271-; HLA-DR-. In a further clinical pilot study (n=5) with 297 ml BM (SD 18.6), the volume reduction protocol concentrated the MSC by a factor of 14: there were 1.08 x 10(2) MSC/ml BM (standard deviation (SD) 1.02 x 10(2)) before concentration, 14.8 x 10(2) MSC/ ml BM (SD 12.4 x 10(2)) after concentration, and on average 296 x 10(2) MSC (SD 248.9 x 10(2), range 86.4-691.5 x 10(2)) were available for MSC therapy. The volume reduction protocol of the closed centrifuge allows for the highest concentration of the MSC, and therefore, is a promising candidate for instant stem cell therapy.

Katayama, N., et al. (2014). "The effects of synthetic oligopeptide derived from enamel matrix derivative on cell proliferation and osteoblastic differentiation of

human mesenchymal stem cells." Int J Mol Sci **15**(8): 14026-14043.

Enamel matrix derivative (EMD) is widely used in periodontal tissue regeneration therapy. However, because the bioactivity of EMD varies from batch to batch, and the use of a synthetic peptide could avoid use from an animal source, a completely synthetic peptide (SP) containing the active component of EMD would be useful. In this study an oligopeptide synthesized derived from EMD was evaluated for whether it contributes to periodontal tissue regeneration. We investigated the effects of the SP on cell proliferation and osteoblast differentiation of human mesenchymal stem cells (MSCs), which are involved in tissue regeneration. MSCs were treated with SP (0 to 1000 ng/mL), to determine the optimal concentration. We examined the effects of SP on cell proliferation and osteoblastic differentiation indicators such as alkaline phosphatase activity, the production of procollagen type 1 C-peptide and osteocalcin, and on mineralization. Additionally, we investigated the role of extracellular signal-related kinases (ERK) in cell proliferation and osteoblastic differentiation induced by SP. Our results suggest that SP promotes these processes in human MSCs, and that ERK inhibitors suppress these effects. In conclusion, SP promotes cell proliferation and osteoblastic differentiation of human MSCs, probably through the ERK pathway.

Kelly, A. M., et al. (2016). "Glucocorticoid Cell Priming Enhances Transfection Outcomes in Adult Human Mesenchymal Stem Cells." Mol Ther **24**(2): 331-341.

Human mesenchymal stem cells (hMSCs) are one of the most widely researched stem cell types with broad applications from basic research to therapeutics, the majority of which require introduction of exogenous DNA. However, safety and scalability issues hinder viral delivery, while poor efficiency hinders nonviral gene delivery, particularly to hMSCs. Here, we present the use of a pharmacologic agent (glucocorticoid) to overcome barriers to hMSC DNA transfer to enhance transfection using three common nonviral vectors. Glucocorticoid priming significantly enhances transfection in hMSCs, demonstrated by a 3-fold increase in efficiency, 4-15-fold increase in transgene expression, and prolonged transgene expression when compared to transfection without glucocorticoids. These effects are dependent on glucocorticoid receptor binding and caused in part by maintenance of normal metabolic function and increased cellular (5-fold) and nuclear (6-10-fold) DNA uptake over hMSCs transfected without glucocorticoids. Results were consistent across five human donors and in cells up to passage five. Glucocorticoid cell priming is a simple and effective technique to significantly enhance nonviral transfection of hMSCs that should enhance their clinical use, accelerate new research, and decrease reliance on early passage cells.

Kemp, K., et al. (2017). "Mesenchymal Stem Cell-Derived Factors Restore Function to Human Frataxin-Deficient Cells." Cerebellum **16**(4): 840-851.

Friedreich's ataxia is an inherited neurological disorder characterised by mitochondrial dysfunction and increased susceptibility to oxidative stress. At present, no therapy has been shown to reduce disease progression. Strategies being trialled to treat Friedreich's ataxia include drugs that improve mitochondrial function and reduce oxidative injury. In addition, stem cells have been investigated as a potential therapeutic approach. We have used siRNA-induced knockdown of frataxin in SH-SY5Y cells as an in vitro cellular model for Friedreich's ataxia. Knockdown of frataxin protein expression to levels detected in patients with the disorder was achieved, leading to decreased cellular viability, increased susceptibility to hydrogen peroxide-induced oxidative stress, dysregulation of key anti-oxidant molecules and deficiencies in both cell proliferation and differentiation. Bone marrow stem cells are being investigated extensively as potential treatments for a wide range of neurological disorders, including Friedreich's ataxia. The potential neuroprotective effects of bone marrow-derived mesenchymal stem cells were therefore studied using our frataxin-deficient cell model. Soluble factors secreted by mesenchymal stem cells protected against cellular changes induced by frataxin deficiency, leading to restoration in frataxin levels and anti-oxidant defences, improved survival against oxidative stress and stimulated both cell proliferation and differentiation down the Schwann cell lineage. The demonstration that mesenchymal stem cell-derived factors can restore cellular homeostasis and function to frataxin-deficient cells further suggests that they may have potential therapeutic benefits for patients with Friedreich's ataxia.

Kermani, A. J., et al. (2008). "Characterization and genetic manipulation of human umbilical cord vein mesenchymal stem cells: potential application in cell-based gene therapy." Rejuvenation Res **11**(2): 379-386.

Stem cells are defined by two main characteristics: self-renewal capacity and commitment to multi-lineage differentiation. The cells have a great therapeutic potential in repopulating damaged tissues as well as being genetically manipulated and used in cell-based gene therapy. Umbilical cord vein is a readily available and inexpensive source of stem cells that are capable of generating various cell types. Despite the recent isolation of human umbilical cord

vein mesenchymal stem cells (UVMSC), the self-renewal capacity and the potential clinical application of the cells are not well known. In the present study, we have successfully isolated and cultured human UVMSCs. Our data further revealed that the isolated cells express the self-renewal genes Oct-4, Nanog, ZFX, Bmi-1, and Nucleostemin; but not Zic-3, Hoxb-4, TCL-1, Tbx-3 and Esrrb. In addition, our immunocytochemistry results revealed the expression of SSEA-4, but not SSEA-3, TRA-1-60, and TRA-1-81 embryonic stem cell surface markers in the cells. Also, we were able to transfect the cells with a reporter, enhanced green fluorescent protein (EGFP), and a therapeutic human brain-derived neurotrophic factor (hBDNF) gene by means of electroporation and obtained a stable cell line, which could constantly express both transgenes. The latter data provide further evidence on the usefulness of umbilical cord vein mesenchymal stem cells as a readily available source of stem cells, which could be genetically manipulated and used in cell-based gene therapy applications.

Khodabandeh, Z., et al. (2017). "Hepatogenic Differentiation Capacity of Human Wharton's Jelly Mesenchymal Stem Cell in a Co-culturing System with Endothelial Cells in Matrigel/collagen Scaffold in the Presence of Fetal Liver Extract." Int J Stem Cells **10**(2): 218-226.

Background: Human Wharton's jelly mesenchymal stem cells (HWJMSCs) isolated from medical waste product can be considered as an accessible source of cells in regenerative medicine. Stem cell-derived hepatocytes have poor function and need appropriate niche to reconstruct the liver structure. Therefore, we attempted to find a novel approach in differentiating HWJMSCs into functional hepatic cells using 3D culture conditions and liver extract that recapitulates vital stage in liver development. Materials and Methods: HWJMSCs were extracted from human Wharton's jelly, characterized by flow cytometry, and differentiated towards osteogenic and adipogenic lineages. HWJMSCs were co-cultured with HUVECs in 3D matrigel/ collagen scaffolds in the presence of fetal liver extract for 14 days. The expression of specific liver genes were evaluated by lectins, PAS and immunocytochemistry. Results: According to flow cytometry data, isolated cells from HWJMSCs were shown to express MSC markers. HWJMSCs co-cultured with HUVECs in matrigel/collagen scaffold with extract expressed albumin, lectins UEA and PNA. Immunohistochemistry of the cells in matrigel/collagen scaffold with or without extract exhibited a positive reaction for CK19. Conclusions: Co-culturing of the HWJMSC/HUVEC in 3D matrigel/collagen scaffold is bimimicary of in vivo cell condition. The results showed that administration of the liver extract in 3D matrigel/collagen culture of HWJMSC/HUVEC can induce hepatocyte marker expression.

Klyushnenkova, E., et al. (2005). "T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression." J Biomed Sci **12**(1): 47-57.

Human mesenchymal stem cells (MSCs) were evaluated for their ability to activate allogeneic T cells in cell mixing experiments. Phenotypic characterization of MSCs by flow cytometry showed expression of MHC Class I alloantigens, but minimal expression of Class II alloantigens and costimulatory molecules, including CD80 (B7-1), CD86 (B7-2), and CD40. T cells purified from peripheral blood mononuclear cells (PBMCs) did not proliferate to allogeneic MSCs. Lack of response was not due to a deficiency of costimulation, since retroviral transduction of MSCs with either B7-1 or B7-2 costimulatory molecules did not result in lymphoproliferation. Although these results suggested that MSCs were immunologically inert or potentially tolerogenic, T cells cultured with MSCs produced IFN-gamma and displayed secondary kinetics to restimulation with PBMCs, indicating alloantigen priming rather than tolerance induction by the MSCs. To determine whether MSCs suppressed alloreactive T cells, MSCs were added to primary mixed lymphocyte reaction (MLR) cultures. MSCs suppressed cell proliferation when added at the initiation of culture or when added to an ongoing MLR culture. Suppression was dose-dependent, genetically unrestricted, and occurred whether or not MSCs were pretreated with IFN-gamma. MSCs in transwell chambers suppressed primary MLR cultures, indicating that suppression was mediated by soluble molecules. Analysis of cytokines in suppressed MLR cultures demonstrated up-regulation of IFN-gamma and IL-10, and down-regulation of TNF-alpha production relative to control cultures. We conclude that MSCs can initiate activation of alloreactive T cells, but do not elicit T cell proliferative responses due to active suppressive mechanisms.

Koh, S. H., et al. (2005). "Co-culture of human CD34+ cells with mesenchymal stem cells increases the survival of CD34+ cells against the 5-aza-deoxycytidine- or trichostatin A-induced cell death." Biochem Biophys Res Commun **329**(3): 1039-1045.

It has been suggested that epigenetic regulation plays an important role in maintaining the stemness and lineage differentiation of hematopoietic stem cells (HSCs), 5-aza-deoxycytidine (aza-D) and Trichostatin A (TSA) being candidate additives for HSC ex vivo expansion. Although they have potent activity to maintain the stemness, they can also cause serious cell death. This study examined the effects of

mesenchymal stem cells (MSCs) on the maintenance of CD34+ cells driven by aza-D and TSA in culture with the combined cytokines of thrombopoietin, flt-3 ligand, stem cell factor, interleukin-3, and interleukin-6. In cultures without MSCs, although aza-D and TSA retained the CD34 frequency 4 to 8 times more than in the cytokines alone, a large portion of cells underwent apoptotic cell death. Consequently, CD34+ cell expansion could not be achieved in any condition without MSCs. In cultures with MSCs, the total cell number was higher in aza-D or TSA than in any conditions in the cultures without MSCs. The CD34 frequency was also similar to the level in the cultures in aza-D or TSA without the MSCs. These results suggest that a co-culture of CD34+ cells with the MSCs might not simply deliver the proliferation signals but also stemness and survival signals, and overlap the action of epigenetic regulators.

Kohli, N., et al. (2015). "An In Vitro Comparison of the Incorporation, Growth, and Chondrogenic Potential of Human Bone Marrow versus Adipose Tissue Mesenchymal Stem Cells in Clinically Relevant Cell Scaffolds Used for Cartilage Repair." Cartilage **6**(4): 252-263.

AIM: To compare the incorporation, growth, and chondrogenic potential of bone marrow (BM) and adipose tissue (AT) mesenchymal stem cells (MSCs) in scaffolds used for cartilage repair. METHODS: Human BM and AT MSCs were isolated, culture expanded, and characterised using standard protocols, then seeded into 2 different scaffolds, Chondro-Gide or Alpha Chondro Shield. Cell adhesion, incorporation, and viable cell growth were assessed microscopically and following calcein AM/ethidium homodimer (Live/Dead) staining. Cell-seeded scaffolds were treated with chondrogenic inducers for 28 days. Extracellular matrix deposition and soluble glycosaminoglycan (GAG) release into the culture medium was measured at day 28 by histology/immunohistochemistry and dimethylmethylene blue assay, respectively. RESULTS: A greater number of viable MSCs from either source adhered and incorporated into Chondro-Gide than into Alpha Chondro Shield. In both cell scaffolds, this incorporation represented less than 2% of the cells that were seeded. There was a marked proliferation of BM MSCs, but not AT MSCs, in Chondro-Gide. MSCs from both sources underwent chondrogenic differentiation following induction. However, cartilaginous extracellular matrix deposition was most marked in Chondro-Gide seeded with BM MSCs. Soluble GAG secretion increased in chondrogenic versus control conditions. There was no marked difference in GAG secretion by MSCs from either cell source. CONCLUSION: Chondro-Gide and Alpha Chondro Shield were permissive to the incorporation and chondrogenic differentiation of human BM and AT MSCs. Chondro-Gide seeded with BM MSCs demonstrated the greatest increase in MSC number and deposition of a cartilaginous tissue.

Koltsova, A. M., et al. (2015). "[Characteristics of New Mesenchymal Stem Cell Line Derived from Human Embryonic Stem Cells]." Tsitologiia **57**(11): 761-770.

New nonimmortalized fibroblast-like cell line SC6-MSC has been obtained from a line of human embryonic stem cells (ESC)--SC6. Numerical and structural karyotypic analysis has shown hypodiploidy karyotypic: 45, X0 in this line. The average cell population doublings time, for SC6-MSC is 26.0 +/- 0.4 h at the 8th passage and 82.0 +/- 9.2 h at the 18th passage. The growth curves showed active proliferation for 8-10 passages with a consequent gradual decrease of proliferative activity, which ended to 20th passage. To determine the line's status, the analysis of the surface markers by flow cytometry was carried out. We have revealed the expression of surface antigens CD44, CD73, CD90, CD105 and HLA-ABC characteristic for human MSC, and the absence of CD34 and HLA-DR expression. However, the level of expression of surface markers CD90 and CD105 was significantly lower in comparison with other MSC lines including the line SC5-MSC derived from the line human ESC-SC5. Immunofluorescence analysis of the expression of the surface markers and transcription factor Oct-4 characteristic for human embryonic stem cells showed the absence of Oct-4 expression and the presence of SSEA-4 and TRA-1-60 expression, which is characteristic for a number of MSC lines with normal karyotype. Immunofluorescence analysis has shown the presence of the markers of early differentiation in the derivates of three germ layers, characteristic for human ESC, which in corresponding microenvironments may allow MSC to be useful for reparation of tissue injures. The directed osteogenic and chondrogenic differentiation of line SC6-MSC has shown. However, no directed adipogenic differentiation of this line has been found. The obtained results with high probability may indicate what alteration of chromosomal and, accordingly, gene balance, in line SC6-MSC with karyotype 45, X0 resulted in decrease in differential potential, in expression CD90, associated in particular with the processes of differentiation and aging of cells.

Komoda, H., et al. (2010). "Reduction of N-glycolylneuraminic acid xenoantigen on human adipose tissue-derived stromal cells/mesenchymal stem cells leads to safer and more useful cell sources for various stem cell therapies." Tissue Eng Part A **16**(4): 1143-1155.

Adipose tissue is an attractive source for somatic stem cell therapy. Currently, human adipose

tissue-derived stromal cells/mesenchymal stem cells (hADSCs/MSCs) are cultured with fetal bovine serum (FBS). Recently, however, not only human embryonic stem cell lines cultured on mouse feeder cells but also bone marrow-derived human MSCs cultured with FBS were reported to express N-glycolylneuraminic acid (Neu5Gc) xenoantigen. Human serum contains high titers of natural preformed antibodies against Neu5Gc. We studied the presence of Neu5Gc on hADSCs/MSCs cultured with FBS and human immune response mediated by Neu5Gc. Our data indicated that hADSCs/MSCs cultured with FBS expressed Neu5Gc and that human natural preformed antibodies could bind to hADSCs/MSCs. However, hADSCs/MSCs express complement regulatory proteins such as CD46, CD55, and CD59 and are largely resistant to complement-mediated cytotoxicity. hADSCs/MSCs cultured with FBS could be injured by antibody-dependent cell-mediated cytotoxicity mechanism. Further, human monocyte-derived macrophages could phagocytose hADSCs/MSCs cultured with FBS and this phagocytic activity was increased in the presence of human serum. Culturing hADSCs/MSCs with heat-inactivated human serum for a week could markedly reduce Neu5Gc on hADSCs/MSCs and prevent immune responses mediated by Neu5Gc, such as binding of human natural preformed antibodies, antibody-dependent cell-mediated cytotoxicity, and phagocytosis. Adipogenic and osteogenic differentiation potentials of hADSCs/MSCs cultured with heat-inactivated human serum were not less than that of those cultured with FBS. For stem cell therapies based on hADSCs/MSCs, hADSCs/MSCs that presented Neu5Gc on their cell surfaces after exposure to FBS should be cleaned up to be rescued from xenogeneic rejection.

Kong, Y., et al. (2014). "Sphingosine-1-phosphate/S1P receptors signaling modulates cell migration in human bone marrow-derived mesenchymal stem cells." Mediators Inflamm **2014**: 565369.

The recruitment of bone marrow-derived mesenchymal stem cells (BMSCs) to damaged tissues and sites of inflammation is an essential step for clinical therapy. However, the signals regulating the motility of these cells are still not fully understood. Sphingosine-1-phosphate (S1P), a bioactive sphingolipid metabolite, is known to have a variety of biological effects on various cells. Here, we investigated the roles of S1P and S1P receptors (S1PRs) in migration of human BMSCs. We found that S1P exerted a powerful migratory action on human BMSCs. Moreover, by employing RNA interference technology and pharmacological tools, we demonstrated that S1PR1 and S1PR3 are responsible for S1P-induced migration of human BMSCs. In contrast, S1PR2 mediates the inhibition of migration. Additionally, we explored the downstream signaling pathway of the S1P/S1PRs axis and found that activation of S1PR1 or S1PR3 increased migration of human BMSCs through a G i /extracellular regulated protein kinases 1/2-(ERK1/2-) dependent pathway, whereas activation of S1PR2 decreased migration through the Rho/Rho-associated protein kinase (ROCK) pathway. In conclusion, we reveal that the S1P/S1PRs signaling axis regulates the migration of human BMSCs via a dual-directional mechanism. Thus, selective modulation of S1PR's activity on human BMSCs may provide an effective approach to immunotherapy or tissue regeneration.

Kono, K., et al. (2015). "Characterization of the cell growth analysis for detection of immortal cellular impurities in human mesenchymal stem cells." Biologicals **43**(2): 146-149.

The analysis of in vitro cell senescence/growth after serial passaging can be one of ways to show the absence of immortalized cells, which are frequently tumorigenic, in human cell-processed therapeutic products (hCTPs). However, the performance of the cell growth analysis for detection of the immortalized cellular impurities has never been evaluated. In the present study, we examined the growth rates of human mesenchymal stem cells (hMSCs, passage 5 (P = 5)) contaminated with various doses of HeLa cells, and compared with that of hMSCs alone. The growth rates of the contaminated hMSCs were comparable to that of hMSCs alone at P = 5, but significantly increased at P = 6 (0.1% and 0.01% HeLa) or P = 7 (0.001% HeLa) within 30 days. These findings suggest that the cell growth analysis is a simple and sensitive method to detect immortalized cellular impurities in hCTPs derived from human somatic cells.

Kupcsik, L., et al. (2009). "Statin-induced calcification in human mesenchymal stem cells is cell death related." J Cell Mol Med **13**(11-12): 4465-4473.

Statins are widely used in clinics to lower cholesterol levels. Recently, they have been shown to positively affect bone formation and bone mass in a rat model. The aim of this study was to investigate the effect of pravastatin, simvastatin and lovastatin on the osteoblastic differentiation of human mesenchymal stem cells (MSCs) in vitro. Cell number, alkaline phosphatase (ALP) activity, matrix mineralization and gene expression pattern were determined. Pravastatin did not affect cell differentiation. Simvastatin and lovastatin enhanced bone morphogenetic protein 2 (BMP-2) mRNA levels. In contrast, ALP activity and mRNA levels were suppressed by statins, as well as the DNA content and cell activity (MTT). An increase in apoptotic events was observed at high concentrations

of statins, along with high Ca-45 incorporation. Lower concentrations of statins did not increase apoptotic staining, but also failed to induce calcification. When statin-induced calcification did occur, the morphology of the deposits was very different from the conventional nodule formation; the calcium was laid down along the membranes of the rounded cells suggesting it was as a result of cell death. Our results indicate that statins are not able to differentiate human MSCs into osteoblasts and that high concentrations of statins (>1 microM) have a cytotoxic effect.

Kurogi, R., et al. (2018). "Inhibition of glioblastoma cell invasion by hsa-miR-145-5p and hsa-miR-31-5p co-overexpression in human mesenchymal stem cells." J Neurosurg: 1-12.

OBJECTIVE Human bone marrow-derived mesenchymal stem cells (hMSCs) show tropism for brain tumors and may be a useful vehicle for drug or gene delivery to malignant gliomas. Recently, some microRNAs (miRNAs) have been shown to suppress the invasiveness of malignant gliomas. METHODS To test their potential to become vehicles for the delivery of miRNA to malignant gliomas, hMSCs were engineered so that hMSC secretion of miRNAs that inhibit glioma cell invasion was enabled without altering the hMSC tropism for glioma cells. RESULTS In coculture, hMSCs cotransfected with hsa-miR-145-5p and -31-5p miRNAs showed markedly reduced invasion by U87 glioma cells in a contact-dependent manner both in vitro and ex vivo, with invasion of hMSCs cotransfected with these 2 miRNAs by the U87 cells reduced to 60.7% compared with control cells. According to a Matrigel invasion assay, the tropism of the hMSCs for U87 cells was not affected. In glioma cell lines U251 and LN229, hMSCs exhibited tropism in vivo, and invasion of hMSCs cotransfected with hsa-miR-145-5p and -31-5p was also significantly less than that of control cells. When U87 cells were coimplanted into the striatum of organotypic rat brain slices with hMSCs cotransfected with hsa-miR-145 and -31-5p, the relative invasive area decreased by 37.1%; interestingly, these U87 cells showed a change to a rounded morphology that was apparent at the invasion front. Whole-genome microarray analysis of the expression levels of 58,341 genes revealed that the co-overexpression of hsa-miR-145-5p and -31-5p downregulated FSCN1 expression in U87 cells. CONCLUSIONS This study demonstrates that miRNA overexpression in hMSCs can alter the function of glioma cells via contact-dependent transfer. Co-overexpression of multiple miRNAs may be a useful and novel therapeutic strategy. The study results suggest that hMSCs can be applied as a delivery vehicle for miRNAs.

Kwon, S. H., et al. (2013). "Modulation of BMP-2-induced chondrogenic versus osteogenic differentiation of human mesenchymal stem cells by cell-specific extracellular matrices." Tissue Eng Part A **19**(1-2): 49-58.

Bone morphogenetic protein-2 (BMP-2) is known to induce both osteogenic and chondrogenic commitment of human mesenchymal stem cells (hMSCs). However, factors influencing BMP-2-dependent chondrogenic and osteogenic differentiation have not been investigated. In this study, we demonstrated that extracellular microenvironments, in the form of cell-derived matrices, play important roles in determining the specific lineage commitment of hMSCs in the presence of BMP-2. Extracellular matrices (ECMs) derived from osteoblasts and chondrocytes were utilized to regulate cell differentiation. Osteogenic and chondrogenic differentiation of hMSCs cultured on the two different cell-derived ECMs were assessed by quantitative real-time-polymerase chain reaction, immunocytochemistry, and western blot analysis. To minimize the effects of the cell-adhesion proteins contained in serum on the ECMs, hMSCs were cultured in serum-free osteogenic or chondrogenic differentiation medium. Fibronectin-, collagen type I-, or collagen type II-coated substrates were utilized as ECM controls. The ECM specific to each cell type promoted lineage-specific commitment of hMSCs in the presence of BMP-2, that is, osteoblast- and chondrocyte-derived ECM promoted osteogenic and chondrogenic commitment, respectively. Therefore, cell-specific ECMs are capable of modulating the BMP-2-induced osteogenic and chondrogenic differentiation of hMSCs.

Lotfinia, M., et al. (2016). "Effect of Secreted Molecules of Human Embryonic Stem Cell-Derived Mesenchymal Stem Cells on Acute Hepatic Failure Model." Stem Cells Dev **25**(24): 1898-1908.

Adult tissue-derived mesenchymal stem cells (MSCs) show tremendous promise for a wide array of therapeutic applications predominantly through paracrine activity. Recent reports showed that human embryonic stem cell (ESC)-derived MSCs are an alternative for regenerative cellular therapy due to manufacturing large quantities of MSCs from a single donor. However, no study has been reported to uncover the secretome of human ESC-MSCs as treatment of an acute liver failure (ALF) mouse model. We demonstrated that human ESC-MSCs showed similar morphology and cell surface markers compared with bone marrow-derived MSCs. ESC-MSCs exhibited a higher growth rate during early in vitro expansion, along with adipogenic and osteogenic differentiation potential. Treatment with ESC-MSC-conditioned medium (CM) led to statistically significant

enhancement of primary hepatocyte viability and increased immunomodulatory interleukin-10 secretion from lipopolysaccharide-induced human blood mononuclear cells. Analysis of the MSCs secretome by a protein array screen showed an association between higher frequencies of secretory proteins such as vascular endothelial growth factor (VEGF) and regulation of cell proliferation, cell migration, the development process, immune system process, and apoptosis. In this thioacetamide-induced mouse model of acute liver injury, we observed that systemic infusion of VEGF led to significant survival. These data have provided the first experimental evidence of the therapeutic potential of human ESC-MSC-derived molecules. These molecules show trophic support to hepatocytes, which potentially creates new avenues for the treatment of ALF, as an inflammatory condition.

Lozito, T. P., et al. (2009). "Human mesenchymal stem cells express vascular cell phenotypes upon interaction with endothelial cell matrix." J Cell Biochem **107**(4): 714-722.

Mesenchymal stem cells (MSCs) are thought to occupy a perivascular niche where they are exposed to signals originating from vascular cells. This study focused on the effects of endothelial cell (EC)-derived signals on MSC differentiation toward vascular cell lineages. Upon co-culture with two types of ECs, macrovascular (macro) ECs and microvascular (micro) ECs, the former caused MSCs to increase expression of both EC and smooth muscle cell (SMC) markers, while the latter induced expression of EC markers only. These marker changes in MSCs were linked to the extracellular matrixes secreted by the ECs (EC-matrix) rather than soluble EC-secreted factors. Beyond enhanced marker expression, EC-matrix also induced functional changes in MSCs indicative of development of a genuine vascular cell phenotype. These included enhanced incorporation into vessels and cytoskeletal localization of vascular SMC-specific contractile elements. The bioactivity of EC-matrix was sensitive to EDTA washes and required sulfated glycosaminoglycans. However, neither soluble VEGF nor substrate surfaces coated with fibronectin, collagen type IV, or laminin recreated the effects of EC-matrix on MSC vascular differentiation. In conclusion, these results identified EC-matrix as a critical regulator of vascular cell differentiation of MSCs. Elucidating these MSC-EC-matrix interactions and identifying the specific EC-matrix components involved will shed light on the perivascular signals seen by MSCs in vivo.

Ma, J., et al. (2017). "Multiphoton Fabrication of Fibronectin-Functionalized Protein Micropatterns: Stiffness-Induced Maturation of Cell-Matrix Adhesions in Human Mesenchymal Stem Cells." ACS Appl Mater Interfaces **9**(35): 29469-29480.

Cell-matrix adhesions are important structures governing the interactions between cells and their microenvironment at the cell-matrix interface. The focal complex (FC) and focal adhesion (FA) have been substantially investigated in conventional planar culture systems using fibroblasts as an in vitro model. However, the formation of more mature types of cell-matrix adhesion in human mesenchymal stem cells (hMSCs), including fibrillar adhesion (FBA) and 3D matrix adhesion (3DMA), have not been fully elucidated. Here we investigate the niche factor(s) that influence(s) the maturation of FBA and 3DMA by using multiphoton fabrication-based micropatterning. First, the bovine serum albumin (BSA)-made protein micropatterns were functionalized by incorporating various concentrations of fibronectin (FN) in fabrication solution. The amount of cross-linked FN is positively correlated with the initial concentration of FN in the reaction liquid, as verified by immunofluorescence staining. On the other hand, the anisotropic FN-functionalized micropatterns were fabricated by varying the length (i.e., in-plane stiffness) and height (i.e., bending stiffness) of micropatterns, respectively. Finally, hMSCs were cultured on these micropatterns for 2 h and 1 day to determine the formation of FBA and 3DMA, respectively, using immunofluorescence staining. Results demonstrated that FN-functionalized micropatterns with high anisotropy in x-y dimension benefit FBA maturation. Furthermore, niche factors such as higher bending and in-plane stiffness and the presence of abundant fibronectin have a positive effect on the maturation of FN-based cell-matrix adhesion. These findings could provide some new perspectives on designing platforms for further cell niche study and rationalizing scaffold design for tissue engineering.

Ma, K., et al. (2011). "Generation of neural stem cell-like cells from bone marrow-derived human mesenchymal stem cells." Neurol Res **33**(10): 1083-1093.

Under appropriate culture conditions, bone marrow (BM)-derived mesenchymal stem cells are capable of differentiating into diverse cell types unrelated to their phenotypical embryonic origin, including neural cells. Here, we report the successful generation of neural stem cell (NSC)-like cells from BM-derived human mesenchymal stem cells (hMSCs). Initially, hMSCs were cultivated in a conditioned medium of human neural stem cells. In this culture system, hMSCs were induced to become NSC-like cells, which proliferate in neurosphere-like structures and express early NSC markers. Like central nervous system-derived NSCs, these BM-derived NSC-like

cells were able to differentiate into cells expressing neural markers for neurons, astrocytes, and oligodendrocytes. Whole-cell patch clamp recording revealed that neuron-like cells, differentiated from NSC-like cells, exhibited electrophysiological properties of neurons, including action potentials. Transplantation of NSC-like cells into mouse brain confirmed that these NSC-like cells retained their capability to differentiate into neuronal and glial cells in vivo. Our data show that multipotent NSC-like cells can be efficiently produced from BM-derived hMSCs in culture and that these cells may serve as a useful alternative to human neural stem cells for potential clinical applications such as autologous neuroreplacement therapies.

Ma, L. J., et al. (2006). "[Effects of human mesenchymal stem cells and fibroblastoid cell line as feeder layers on expansion of umbilical cord blood CD34(+) cells in vitro]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **14**(5): 949-954.

To investigate the effects of human mesenchymal stem cells (MSC) and human fibroblastoid cell line (HFCL) as feeder layer on expansion of umbilical cord blood CD34(+) cells in vitro, (60)Co gamma-ray irradiated MSC and HFCL were used as feeder layer to expand cord blood CD34(+) cells in culture. The efficiencies of MSC and HFCL on expansion of CD34(+) cells in culture with or without cytokines were compared. The results showed that no matter whether cytokines (rhFL, rhSCF, rhTPO) were added, the proliferation of nucleated cells after expansion for 12 days in HFCL group was statistically higher than that in MSC group, i.e. with cytokines (9797 +/- 361)% vs (7061 +/- 418)%; without cytokines (5305 +/- 354)% vs (1992 +/- 247)%, when the cell numbers at day 0 was accounted as 100%), $P < 0.01$. The proliferation of propagated CD34(+) cells between MSC group and HFCL without addition of cytokines was not statistically different (820 +/- 191)% vs (825 +/- 305)%, $P > 0.05$. However, in the presence of cytokines, the propagating rate of MSC group was lower than that of HFCL group (939 +/- 212)% vs (1617 +/- 222)%, $P < 0.01$. MSC was better than HFCL in maintaining the LTC-IC of UCB CD34(+) cells, i.e. the number of CFU-GM colonies in the fifth week was (129.95 +/- 8.73) /10(5) seeded cells vs (89.81 +/- 10.29) colonies/10(5) cells, $P < 0.05$; with addition of cytokines, the effect was more obvious, i.e. the number of CFU-GM colonies in the fifth week (192.93 +/- 4.95)/10(5) seeded cells vs (90.47 +/- 14.28) colonies/10(5) seeded cells, $P < 0.01$. MSC mixed with a certain proportion of HFCL facilitated maintaining the LTC-IC of UCB CD34(+) cells. When the proportion was 4:1, the number of CFU-GM colonies was the highest (186.89 +/- 11.11)/10(5) seeded cells, which was higher than that of both 3:2 group [(138.92 +/- 14.84) colonies/10(5) seeded cells] and MSC only group, i.e. (64.63 +/- 6.11) colonies/10(5) seeded cells, both $P < 0.01$. It is concluded that HFCL is better than MSC in maintaining the expansion of CD34(+) cells and cytokines can enhance this effect, while MSC are stronger than HFCL in maintaining the LTC-IC of UCB CD34(+) cells in vitro. MSC with addition of a certain proportion of HFCL can significantly enhance the efficiency of CD34(+) cell expansion.

Maccario, R., et al. (2005). "Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype." Haematologica **90**(4): 516-525.

BACKGROUND AND OBJECTIVES: Experimental evidence and preliminary clinical studies have demonstrated that human mesenchymal stem cells (MSC) have an important immune modulatory function in the setting of allogeneic hematopoietic stem cell (HSC) transplantation. We extended the evaluation of mechanisms responsible for the immune regulatory effect derived from the interaction of human MSC with cells involved in alloantigen-specific immune response in mixed lymphocyte culture (MLC). DESIGN AND METHODS: Dendritic cell (DC) differentiation, T- and natural killer (NK)-lymphocyte expansion, alloantigen-specific cytotoxic activity and differentiation of CD4+ T-cell subsets co-expressing CD25 and/or CTLA4 molecules were assessed, comparing the effect observed using third-party MSC with that obtained employing MSC autologous to the MLC responder. RESULTS: We found that human MSC strongly inhibit alloantigen-induced DC1 differentiation, down-regulate alloantigen-induced lymphocyte expansion, especially that of CD8+ T cells and of NK lymphocytes, decrease alloantigen-specific cytotoxic capacity mediated by either cytotoxic T lymphocytes or NK cells and favor the differentiation of CD4+ T-cell subsets co-expressing CD25 and/or CTLA4. More effective suppressive activity on MLC-induced T-cell activation was observed when MSC were third-party, rather than autologous, with respect to MLC-responder cells. INTERPRETATION AND CONCLUSIONS: Our results strongly suggest that MSC-mediated inhibition of alloantigen-induced DC1 differentiation and preferential activation of CD4+ CD25+ T-cell subsets with presumed regulatory activity represent important mechanisms contributing to the immunosuppressive activity of MSC. Collectively, these data provide immunological support for the use of MSC to prevent immune complications related to both HSC and solid organ transplantation and to the theory that MSC are universal suppressors of immune reactivity.

Madsen, S. D., et al. (2017). "Decoy TRAIL receptor CD264: a cell surface marker of cellular aging for human bone marrow-derived mesenchymal stem cells." Stem Cell Res Ther **8**(1): 201.

BACKGROUND: Mesenchymal stem cells (MSCs) are a mixture of progenitors that are heterogeneous in their regenerative potential. Development of MSC therapies with consistent efficacy is hindered by the absence of an immunophenotype of MSC heterogeneity. This study evaluates decoy TRAIL receptor CD264 as potentially the first surface marker to detect cellular aging in heterogeneous MSC cultures. METHODS: CD264 surface expression, regenerative potential, and metrics of cellular aging were assessed in vitro for marrow MSCs from 12 donors ages 20-60 years old. Male and female donors were age matched. Expression of CD264 was compared with that of p16, p21, and p53 during serial passage of MSCs. RESULTS: When CD264(+) cell content was 20% to 35%, MSC cultures from young (ages 20-40 years) and older (ages 45-60 years) donors proliferated rapidly and differentiated extensively. Older donor MSCs containing < 35% CD264(+) cells had a small size and negligible senescence despite the donor's advanced chronological age. Above the 35% threshold, CD264 expression inversely correlated with proliferation and differentiation potential. When CD264(+) cell content was 75%, MSCs were enlarged and mostly senescent with severely compromised regenerative potential. There was no correlation of the older donors' chronological age to either CD264(+) cell content or the regenerative potential of the donor MSCs. CD264 was upregulated after p53 and had a similar expression profile to that of p21 during serial passage of MSCs. No sex-linked differences were detected in this study. CONCLUSIONS: These results suggest that CD264 is a surface marker of cellular age for MSCs, not the chronological age of the MSC donor. CD264 is first upregulated in MSCs at an intermediate stage of cellular aging and remains upregulated as aging progresses towards senescence. The strong inverse correlation of CD264(+) cell content to the regenerative potential of MSCs has possible application to assess the therapeutic potential of patient MSCs, standardize the composition and efficacy of MSC therapies, and facilitate aging research on MSCs.

Maeda, S., et al. (2011). "Shrinkage-free preparation of scaffold-free cartilage-like disk-shaped cell sheet using human bone marrow mesenchymal stem cells." J Biosci Bioeng **111**(4): 489-492.

Aiming for the clinical application of cartilage regeneration, a culture method for mesenchymal stem cells (MSCs) derived from human bone marrow to obtain scaffold-free cartilage-like disk-shaped sheet of uniform sizes without the shrinkage was investigated. A disk-shaped cell sheet having the same diameter as that of the membrane without the shrinkage was formed after the cultivation of MSCs (18.6 x 10(5)cells/well) for 3 weeks in a cell culture insert (CCI) containing a flat membrane whose porosity was 12%, while 6.2 and 31.0 x 10(5)MSCs/well, respectively, resulted in the shrinkage of the aggregate and the hole formation in the center part of the sheet. Cell aggregates shrunk also in a 96-well plate and CCIs having lower porosity. The disk-shaped cell sheet showed the comparable thickness (1.2mm) and sulfated glycosaminoglycan (sGAG) density to those of the pellet formed in a pellet culture. The gene expression levels of aggrecan and type II collagen in the disk-shaped cell sheet were not lower than those in the pellet. In conclusion, the usage of CCI having 12% porosity and 18.6 x 10(5)MSCs/well could avoid the shrinkage from the formation of the scaffold-free cartilage-like disk-shaped cell sheet.

Mahalingaiah, P. K., et al. (2015). "Chronic oxidative stress leads to malignant transformation along with acquisition of stem cell characteristics, and epithelial to mesenchymal transition in human renal epithelial cells." J Cell Physiol **230**(8): 1916-1928.

Oxidative injury to cellular macromolecules has been suggested as a common pathway shared by multiple etiological factors for kidney cancer. Whether the chronic oxidative stress alone is sufficient to induce malignant transformation in human kidney cells is not clear. Therefore, the objective of this study was to evaluate the effect of H2O2-induced chronic oxidative stress on growth, and malignant transformation of HK-2 normal kidney epithelial cells. This study revealed that chronic oxidative stress causes increased growth and neoplastic transformation in normal kidney epithelial cells at non-cytotoxic dose and increased adaptation to cytotoxic level. This was confirmed by gene expression changes, cell cycle analysis, anchorage independent growth assay and in vivo tumorigenicity in nude mice. Stem cells characteristics as revealed by up-regulation of stem cell marker genes, and morphological changes indicative of EMT with up regulation of mesenchymal markers were also observed in cells exposed to chronic oxidative stress. Antioxidant NAC did not reverse the chronic oxidative stress-induced growth, and adaptation suggesting that perturbed biological function in these cells are permanent. Partial reversal of oxidative stress-induced growth, and adaptation by silencing of Oct 4 and Snail1, respectively, suggest that these changes are mediated by acquisition of stem cell and EMT characteristics. In summary, this study for the first time suggests that chronic exposure to elevated levels of oxidative stress

is sufficient to induce malignant transformation in kidney epithelial cells through acquisition of stem cell characteristics. Additionally, the EMT plays an important role in increased adaptive response of renal cells to oxidative stress.

Maitra, B., et al. (2004). "Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation." Bone Marrow Transplant **33**(6): 597-604.

Bone marrow-derived mesenchymal stem cells (MSCs) are known to interact with hematopoietic stem cells (HSCs) and immune cells, and represent potential cellular therapy to enhance allogeneic hematopoietic engraftment and prevent graft-versus-host disease (GVHD). We investigated the role of human MSCs in NOD-SCID mice repopulation by unrelated human hematopoietic cells and studied the immune interactions between human MSCs and unrelated donor blood cells in vitro. When hematopoietic stem cell numbers were limited, human engraftment of NOD-SCID mice was observed only after coinfusion of unrelated human MSCs, but not with coinfusion of mouse mesenchymal cell line. Unrelated human MSCs did not elicit T-cell activation in vitro and suppressed T-cell activation by Tuberculin and unrelated allogeneic lymphocytes in a dose-dependent manner. Cell-free MSC culture supernatant, mouse stromal cells and human dermal fibroblasts did not elicit this effect. These preclinical data suggest that unrelated, human bone marrow-derived, culture-expanded MSCs may improve the outcome of allogeneic transplantation by promoting hematopoietic engraftment and limiting GVHD and their therapeutic potential should be tested in clinic.

Majumdar, M. K., et al. (2003). "Characterization and functionality of cell surface molecules on human mesenchymal stem cells." J Biomed Sci **10**(2): 228-241.

We have characterized adhesion molecules on the surface of multipotential human mesenchymal stem cells (hMSCs) and identified molecules whose ligands are present on mature hematopoietic cells. Flow cytometric analysis of hMSCs identified the expression of integrins: alpha1, alpha2, alpha3, alpha5, alpha6, alphav, beta1, beta3, and beta4, in addition to ICAM-1, ICAM-2, VCAM-1, CD72, and LFA-3. Exposure of hMSCs to IL-1alpha, TNFalpha or IFNgamma up-modulated ICAM-1 surface expression, whereas only IFNgamma increased both HLA-class I and -class II molecules on the cell surface. Whole cell-binding assays between the hMSCs and hematopoietic cell lines showed that T lymphocytic lines bound hMSCs with higher affinity than lines of either B lymphocytes or those of myeloid lineage. Experiments using autologous T lymphocytes isolated from peripheral blood mononuclear cells showed that hMSCs exhibited increased affinity for activated T-lymphocytes compared to resting T cells by quantitative whole cell binding and rosetting assays. Flow cytometric analysis of rosetted cells demonstrated that both CD4+ and CD8+ cells bound to hMSCs. To determine the functional significance of these findings, we tested the ability of hMSCs to present antigen to T lymphocytes. hMSCs pulsed with tetanus toxoid stimulated proliferation and cytokine production (IL-4, IL-10, and IFNgamma) in a tetanus-toxoid-specific T cell line. Maximal cytokine production correlated with maximal antigen-dependent proliferation. These data demonstrate physiological outcome as a consequence of interactions between hMSCs and human hematopoietic lineage cells, suggesting a role for hMSCs in vivo to influence both hematopoietic and immune function(s).

Maleki, M., et al. (2014). "Comparison of mesenchymal stem cell markers in multiple human adult stem cells." Int J Stem Cells **7**(2): 118-126.

OBJECTIVES: Mesenchymal stem cells (MSCs) are adult stem cells which identified by adherence to plastic, expression of cell surface markers including CD44, CD90, CD105, CD106, CD166, and Stro-1, lack of the expression of hematopoietic markers, no immunogenic effect and replacement of damaged tissues. These properties led to development of progressive methods to isolation and characterization of MSCs from various sources for therapeutic applications in regenerative medicine. METHODS: We isolated MSC-like cells from testis biopsies, ovary, hair follicle and umbilical cord Wharton's jelly and investigated the expression of specific cell surface antigens using flow cytometry in order to verify stemness properties of these cells. RESULTS: All four cell types adhered to plastic culture flask a few days after primary culture. All our cells positively expressed common MSC- specific cell surface markers. Moreover, our results revealed the expression of CD19and CD45 antigens in these cells. CONCLUSION: According to our results, high expression of CD44 in spermatogonial stem cells (SSCs), hair follicle stem cells (HFSCs),granulosa cells (GCs)and Wharton's jelly-MSCs (WJ-MSCs)may help them to maintain stemness properties. Furthermore, we suggest that CD105+SSCs, HFSCs and WJ-MSCs revealed the osteogenic potential of these cells. Moreover, high expression of CD90 in SSCs and HFSCs may associate to higher growth and differentiation potential of these cells. Further, the presence of CD19 on SSCs and GCs may help them to efficiency in response to trans-membrane signals. Thus, these four types of MSCs may be useful in clinical applications and cell therapy.

Mao, Y., et al. (2017). "Cell type-specific extracellular matrix guided the differentiation of human mesenchymal stem cells in 3D polymeric scaffolds." J Mater Sci Mater Med **28**(7): 100.

The tissue microenvironment has profound effects on tissue-specific regeneration. The 3-dimensional extracellular matrix (ECM) niche influences the linage-specific differentiation of stem cells in tissue. To understand how ECM guides tissue-specific regeneration, we established a series of 3D composite scaffolds containing ECMs derived from different primary cells isolated from a single animal species and assessed their impact on the differentiation of human mesenchymal stem cells (hMSCs). Synthetic microfiber scaffolds (fiber mats) were fabricated by electrospinning tyrosine-derived polycarbonates (pDTEC). The bovine primary fibroblasts, chondrocytes and osteoblasts cultured on the fiber mats produced and assembled their ECMs, infiltrating the pores of the fibrous scaffold. The composite scaffolds were decellularized to remove cellular components, preserve ECM and minimally affect polymer integrity. Characterization of the ECMs derived from different primary cells in the composite scaffolds showed overlapping but distinct compositions. The chondrogenic and osteogenic differentiation of hMSCs on the different composite scaffolds were compared. Our results showed that ECM derived from chondrocytes cultured in synthetic fiber mats promoted the chondrogenic differentiation of hMSC in the presence or absence of soluble inducing factors. ECM derived from co-culture of osteoblasts and chondrocytes promoted osteogenic differentiation in hMSCs better than ECM derived from chondrocytes. This study demonstrated that decellularized ECMs derived from different cell types formed within synthetic fiber scaffolds guide the tissue-specific differentiation of hMSCs. These composite scaffolds may be developed into models to study the mechanisms of ECM-induced tissue regeneration.

Maqbool, M., et al. (2011). "Human mesenchymal stem cells protect neutrophils from serum-deprived cell death." Cell Biol Int **35**(12): 1247-1251.

We have previously shown that human MSC (mesenchymal stem cells) inhibit the proliferation of most of the immune cells. However, there are innate immune cells such as neutrophils and other PMN (polymorphonuclear) cells that do not require an extensive proliferation prior to their effector function. In this study, the effect of MSC on neutrophils in the presence of complete and serum-deprived culture media was investigated. In the presence of MSC, the viability of neutrophils increase as measured in 24 h of incubation at various supplementation of serum concentration. We have utilized Annexin V and PI (propidium iodide) staining to confirm whether the enhancement of neutrophil's viability is due to a reduction in PCD (programmed cell death). MSC significantly rescue neutrophils from apoptosis at 1, 5 and 10% of FBS (fetal bovine serum) supplementation. The fractions of viable and dead cells were increased and decreased respectively in the presence of MSC. Our results indicate MSC rescue neutrophils from nutrient- or serum-deprived cell death. However, whether this effect is exerted through a specific signalling pathway or confining neutrophils in resting state by MSC requires further investigation.

Marrelli, M., et al. (2013). "Cells isolated from human periapical cysts express mesenchymal stem cell-like properties." Int J Biol Sci **9**(10): 1070-1078.

We provide a detailed description of mesenchymal stem cells (MSCs) isolated from human periapical cysts, which we have termed hPCy-MSCs. These cells have a fibroblast-like shape and adhere to tissue culture plastic surfaces. hPCy-MSCs possess high proliferative potential and self-renewal capacity properties. We characterised the immunophenotype of hPCy-MSCs (CD73(+), CD90(+), CD105(+), CD13(+), CD29(+), CD44(+), CD45(-), STRO-1(+), CD146(+)) by flow cytometry and immunofluorescence. hPCy-MSCs possess the potential to differentiate into osteoblast- and adipocyte-like cells in vitro. Multi-potentiality was evaluated with culture-specific staining and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis for osteo/odontogenic and adipogenic markers. This is the first report to indicate that human periapical cysts contain cells with MSC-like properties. Taken together, our findings indicate that human periapical cysts could be a rich source of MSCs.

McFadden, T. M., et al. (2013). "The delayed addition of human mesenchymal stem cells to pre-formed endothelial cell networks results in functional vascularization of a collagen-glycosaminoglycan scaffold in vivo." Acta Biomater **9**(12): 9303-9316.

This paper demonstrates a method to engineer, in vitro, a nascent microvasculature within a collagen-glycosaminoglycan scaffold with a view to overcoming the major issue of graft failure due to avascular necrosis of tissue-engineered constructs. Human umbilical vein endothelial cells (ECs) were cultured alone and in various co-culture combinations with human mesenchymal stem cells (MSCs) to determine their vasculogenic abilities in vitro. Results demonstrated that the delayed addition of MSCs to pre-formed EC networks, whereby MSCs act as pericytes to the nascent vessels, resulted in the best developed vasculature. The results also demonstrate that the crosstalk between ECs and MSCs during microvessel

formation occurs in a highly regulated, spatio-temporal fashion, whereby the initial seeding of ECs results in platelet derived growth factor (PDGF) release; the subsequent addition of MSCs 3 days later leads to a cessation in PDGF production, coinciding with increased vascular endothelial cell growth factor expression and enhanced vessel formation. Functional assessment of these pre-engineered constructs in a subcutaneous rat implant model demonstrated anastomosis between the in vitro engineered vessels and the host vasculature, with significantly increased vascularization occurring in the co-culture group. This study has thus provided new information on the process of in vitro vasculogenesis within a three-dimensional porous scaffold for tissue engineering and demonstrates the potential for using these vascularized scaffolds in the repair of critical sized bone defects.

Medda, R., et al. (2014). "Investigation of early cell-surface interactions of human mesenchymal stem cells on nanopatterned beta-type titanium-niobium alloy surfaces." Interface Focus **4**(1): 20130046.

Multi-potent adult mesenchymal stem cells (MSCs) derived from bone marrow have therapeutic potential for bone diseases and regenerative medicine. However, an intrinsic heterogeneity in their phenotype, which in turn results in various differentiation potentials, makes it difficult to predict the response of these cells. The aim of this study is to investigate initial cell-surface interactions of human MSCs on modified titanium alloys. Gold nanoparticles deposited on beta-type Ti-40Nb alloys by block copolymer micelle nanolithography served as nanotopographical cues as well as specific binding sites for the immobilization of thiolated peptides present in several extracellular matrix proteins. MSC heterogeneity persists on polished and nanopatterned Ti-40Nb samples. However, cell heterogeneity and donor variability decreased upon functionalization of the gold nanoparticles with cyclic RGD peptides. In particular, the number of large cells significantly decreased after 24 h owing to the arrangement of cell anchorage sites, rather than peptide specificity. However, the size and number of integrin-mediated adhesion clusters increased in the presence of the integrin-binding peptide (cRGDfK) compared with the control peptide (cRADfK). These results suggest that the use of integrin ligands in defined patterns could improve MSC-material interactions, not only by regulating cell adhesion locally, but also by reducing population heterogeneity.

Menge, T., et al. (2013). "Human mesenchymal stem cells inhibit endothelial proliferation and angiogenesis via cell-cell contact through modulation of the VE-Cadherin/beta-catenin signaling pathway." Stem Cells Dev **22**(1): 148-157.

Over the past 10 years, a great deal has been learned about the fundamental biology and therapeutic application of bone marrow-derived human mesenchymal stem cells (MSCs). Intravenous administration of these cells is the preferred route for therapeutic delivery of MSCs. Vascular endothelial cells (ECs) are the first cell type that MSCs encounter following IV administration. However, little is known about the biological consequences of interactions between MSCs and ECs, and if any therapeutic benefit results from this interaction. We show that MSCs exert potent stabilizing effects on ECs using an in vitro coculture system. Such effects include decreased EC proliferation and the reduction of EC vascular network formation in matrigel. Interestingly, these effects appear to require EC-MSC contact and result in enhanced colocalization of VE-Cadherin and beta-catenin at the cell membrane. Disruption of the VE-Cadherin/beta-catenin interaction abrogates the observed effects. As a functional in vivo correlate, we show that intravenously administered MSCs strongly inhibit angiogenesis in a matrigel plug assay. Taken together, these results identify a novel mechanism of action of MSCs that involves a contact-dependent EC interaction. These findings are relevant to intravenous use of MSCs and provide insight into further optimizing therapeutic strategies involving MSCs.

Merceron, C., et al. (2011). "The effect of two- and three-dimensional cell culture on the chondrogenic potential of human adipose-derived mesenchymal stem cells after subcutaneous transplantation with an injectable hydrogel." Cell Transplant **20**(10): 1575-1588.

Articular cartilage is an avascular tissue composed of chondrocytes, a unique cell type responsible for abundant matrix synthesis and maintenance. When damaged, it never heals spontaneously under physiological circumstances. Therefore, the delivery of mesenchymal stem cells using hydrogel has been considered for cartilage repair. This study aims at investigating the influence of in vitro chondrogenic differentiation of human adipose tissue-derived stem cells (hATSCs) on in vivo cartilage formation when associated with a cellulose-based self-setting hydrogel (Si-HPMC). hATSCs were characterized for their proliferation, surface marker expression, and multipotency. The in vitro chondrogenic potential of hATSCs cultured within Si-HPMC in control or chondrogenic medium was evaluated by measuring COL2A1, ACAN, SOX9, and COMP expression by real-time PCR. Alcian blue and type II collagen staining were also performed. To determine whether in vitro chondrogenically differentiated hATSCs may give rise to cartilage in vivo, cells differentiated as a monolayer or in pellets

were finally associated with Si-HPMC and implanted subcutaneously into nude mice. Cartilage formation was assessed histologically by alcian blue and type II collagen staining. Our data demonstrate that hATSCs exhibited proliferation and self-renewal. hATSCs also expressed typical stem cell surface markers and were able to differentiate towards the adipogenic, osteogenic, and chondrogenic lineages. Real-time PCR and histological analysis indicated that Si-HPMC enabled chondrogenic differentiation of hATSCs in inductive medium, as demonstrated by increased expression of chondrogenic markers. In addition, histological analysis of implants showed that chondrogenically differentiated hATSCs (monolayers or pellets) have the ability to form cartilaginous tissue, as indicated by the presence of sulphated glycosaminoglycans and type II collagen. This study therefore suggests that an in vitro induction of hATSCs in 2D was sufficient to obtain cartilaginous tissue formation in vivo. Si-HPMC associated with autologous hATSCs could thus be a significant tool for regenerative medicine in the context of cartilage damage.

Milanesi, A., et al. (2012). "beta-Cell regeneration mediated by human bone marrow mesenchymal stem cells." PLoS One **7**(8): e42177.

Bone marrow mesenchymal stem cells (BMSCs) have been shown to ameliorate diabetes in animal models. The mechanism, however, remains largely unknown. An unanswered question is whether BMSCs are able to differentiate into beta-cells in vivo, or whether BMSCs are able to mediate recovery and/or regeneration of endogenous beta-cells. Here we examined these questions by testing the ability of hBMSCs genetically modified to transiently express vascular endothelial growth factor (VEGF) or pancreatic-duodenal homeobox 1 (PDX1) to reverse diabetes and whether these cells were differentiated into beta-cells or mediated recovery through alternative mechanisms. Human BMSCs expressing VEGF and PDX1 reversed hyperglycemia in more than half of the diabetic mice and induced overall improved survival and weight maintenance in all mice. Recovery was sustained only in the mice treated with hBMSCs-VEGF. However, de novo beta-cell differentiation from human cells was observed in mice in both cases, treated with either hBMSCs-VEGF or hBMSCs- PDX1, confirmed by detectable level of serum human insulin. Sustained reversion of diabetes mediated by hBMSCs-VEGF was secondary to endogenous beta-cell regeneration and correlated with activation of the insulin/IGF receptor signaling pathway involved in maintaining beta-cell mass and function. Our study demonstrated the possible benefit of hBMSCs for the treatment of insulin-dependent diabetes and gives new insight into the mechanism of beta-cell recovery after injury mediated by hBMSC therapy.

Minguell, J. J., et al. (2005). "Nonstimulated human uncommitted mesenchymal stem cells express cell markers of mesenchymal and neural lineages." Stem Cells Dev **14**(4): 408-414.

Ex vivo cultures of human bone marrow-derived mesenchymal stem cells (MSCs) contain subsets of progenitors exhibiting dissimilar properties. One of these subsets comprises uncommitted progenitors displaying distinctive features, such as morphology, a quiescent condition, growth factor production, and restricted tissue biodistribution after transplantation. In this study, we assessed the competence of these cells to express, in the absence of differentiation stimuli, markers of mesoderm and ectodermic (neural) cell lineages. Fluorescence microscopy analysis showed a unique pattern of expression of osteogenic, chondrogenic, muscle, and neural markers. The depicted "molecular signature" of these early uncommitted progenitors, in the absence of differentiation stimuli, is consistent with their multipotentiality and plasticity as suggested by several in vitro and in vivo studies.

Moghadasali, R., et al. (2013). "Mesenchymal stem cell-conditioned medium accelerates regeneration of human renal proximal tubule epithelial cells after gentamicin toxicity." Exp Toxicol Pathol **65**(5): 595-600.

Bone marrow-derived mesenchymal stem cells (MSCs) have the capacity to regenerate renal tubule epithelia and repair renal function without fusing with resident tubular cells. The goal of the present project was to investigate the role of MSCs secreted cytokines on tubule cell viability and regeneration after a toxic insult, using a conditionally immortalized human proximal tubule epithelial cell (ciPTEC) line. Gentamicin was used to induce nephrotoxicity, and cell viability and migration were studied in absence and presence of human MSC-conditioned medium (hMSC-CM) i.e. medium containing soluble factors produced and secreted by MSCs. Exposure of ciPTEC to 0-3000 mug/ml gentamicin for 24 h caused a significant dose-dependent increase in cell death. We further demonstrated that the nephrotoxic effect of 2000 mug/ml gentamicin was recovered partially by exposing cells to hMSC-CM. Moreover, exposure of ciPTEC to gentamicin (1500-3000 mug/ml) for 7 days completely attenuated the migratory capacity of the cells. In addition, following scrape-wounding, cell migration of both untreated and gentamicin-exposed cells was increased in the presence of hMSC-CM, as compared to exposures to normal medium, indicating

improved cell recovery. Our data suggest that cytokines secreted by MSCs stimulate renal tubule cell regeneration after nephrotoxicity.

Montanucci, P., et al. (2016). "Restoration of t cell substes of patients with type 1 diabetes mellitus by microencapsulated human umbilical cord Wharton jelly-derived mesenchymal stem cells: An in vitro study." Clin Immunol **163**: 34-41.

Human umbilical cord Wharton jelly-derived mesenchymal stem cells (hUCMS) might apply to treating chronic autoimmune disorders, as already shown for Sjogren's syndrome, including type 1 diabetes mellitus (T1D). Since naked hUCMS grafts encountered restraints, we enveloped hUCMS, within immunoisolatory microcapsules (CpS-hUCMS), made of our endotoxin-free, clinical grade alginate. We then examined the vitro effects of interferon (IFN)-gamma-pretreated CpS-hUCMS on Th17 and Treg of T1D patients (n=15) and healthy controls (n=10). Peripheral blood mononuclear cells (PBMCs) were co-cultured with PBMC/CpS-hUCMS: lymphocyte proliferation was assessed by carboxyfluorescein succinimidyl esther (CFSE) dilution assay, and phenotypic analysis of regulatory and effector Tc was also performed. Cytokine expression was performed by bead array and qPCR on IFN-gamma-pretreated hUCMS before PBMCs co-culture. CpS-hUCMS restored a correct Treg/Th17 ratio, relevant to the T1D disease process. In summary, we have preliminarily developed a new biohybrid system, associated with immunoregulatory properties, that is ready for in vivo application.

Morandi, F., et al. (2008). "Immunogenicity of human mesenchymal stem cells in HLA-class I-restricted T-cell responses against viral or tumor-associated antigens." Stem Cells **26**(5): 1275-1287.

Human mesenchymal stem cells (MSC) are immunosuppressive and poorly immunogenic but may act as antigen-presenting cells (APC) for CD4(+) T-cell responses; here we have investigated their ability to serve as APC for in vitro CD8(+) T-cell responses. MSC pulsed with peptides from viral antigens evoked interferon (IFN)-gamma and Granzyme B secretion in specific cytotoxic T lymphocytes (CTL) and were lysed, although with low efficiency. MSC transfected with tumor mRNA or infected with a viral vector carrying the Hepatitis C virus NS3Ag gene induced cytokine release but were not killed by specific CTL, even following pretreatment with IFN-gamma. To investigate the mechanisms involved in MSC resistance to CTL-mediated lysis, we analyzed expression of human leukocyte antigen (HLA) class I-related antigen-processing machinery (APM) components and of immunosuppressive HLA-G molecules in MSC. The LMP7, LMP10, and ERp57 components were not expressed and the MB-1 and zeta molecules were downregulated in MSC either unmanipulated or pretreated with IFN-gamma. Surface HLA-G was constitutively expressed on MSC but was not involved in their protection from CTL-mediated lysis. MSC supernatants containing soluble HLA-G (sHLA-G) inhibited CTL-mediated lysis, whereas those lacking sHLA-G did not. The role of sHLA-G in such inhibition was unambiguously demonstrated by partial restoration of lysis following sHLA-G depletion from MSC supernatants. In conclusion, human MSC can process and present HLA class I-restricted viral or tumor antigens to specific CTL with a limited efficiency, likely because of some defects in APM components. However, they are protected from CTL-mediated lysis through a mechanism that is partly sHLA-G-dependent.

Moreno, R., et al. (2017). "Human Menstrual Blood-Derived Mesenchymal Stem Cells as Potential Cell Carriers for Oncolytic Adenovirus." Stem Cells Int **2017**: 3615729.

Antitumor efficacy of systemically administered oncolytic adenoviruses (OAdv) is limited due to diverse factors such as liver sequestration, neutralizing interactions in blood, elimination by the immune system, and physical barriers in tumors. It is therefore of clinical relevance to improve OAdv bioavailability and tumor delivery. Among the variety of tumor-targeting strategies, the use of stem cells and specifically bone marrow-derived mesenchymal stem cells (BM-MSCs) is of particular interest due to their tumor tropism and immunomodulatory properties. Nonetheless, the invasive methods to obtain these cells, the low number of MSCs present in the bone marrow, and their restricted in vitro expansion represent major obstacles for their use in cancer treatments, pointing out the necessity to identify an alternative source of MSCs. Here, we have evaluated the use of menstrual blood-derived mesenchymal stem cells (MenSCs) as cell carriers for regional delivery of an OAdv in the tumor. Our results indicate that MenSCs can be isolated without invasive methods, they have an increased proliferation rate compared to BM-MSCs, and they can be efficiently infected with different serotype 5-based capsid-modified adenoviruses, leading to viral replication and release. In addition, our in vivo studies confirmed the tumor-homing properties of MenSCs after regional administration.

Moshtagh, P. R., et al. (2013). "Differentiation of human adipose-derived mesenchymal stem cell into insulin-producing cells: an in vitro study." J Physiol Biochem **69**(3): 451-458.

Stem cells with the ability to differentiate into insulin-producing cells (IPCs) are becoming the most

promising therapy for diabetes mellitus and reduce the major limitations of availability and allogeneic rejection of beta cell transplantations. Mesenchymal stem cells (MSCs) are pluripotent stromal cells with the ability to proliferate and differentiate into a variety of cell types including endocrine cells of the pancreas. This study sought to inspect the in vitro differentiation of human adipose-derived tissue stem cells into IPCs which could provide an abundant source of cells for the purpose of diabetic cell therapy in addition to avoid immunological rejection. Adipose-derived MSCs were obtained from liposuction aspirates and induced to differentiate into insulin-secreting cells under a three-stage protocol based on a combination of low-glucose DMEM medium, beta-mercaptoethanol, and nicotinamide for pre-induction and high-glucose DMEM, beta-mercaptoethanol, nicotinamide, and exendin-4 for induction stages of differentiation. Differentiation was evaluated by the analysis of morphology, dithizone staining, RT-PCR, and immunocytochemistry. Morphological changes including typical islet-like cell clusters were observed by phase-contrast microscope at the end of differentiation protocol. Based on dithizone staining, differentiated cells were positive and undifferentiated cells were not stained. Furthermore, RT-PCR results confirmed the expression of insulin, PDX1, Ngn3, PAX4, and GLUT2 in differentiated cells. Moreover, insulin production by the IPCs was confirmed by immunocytochemistry analysis. It is concluded that adipose-derived MSCs could differentiate into insulin-producing cells in vitro.

Moslem, M., et al. (2013). "Therapeutic potential of human induced pluripotent stem cell-derived mesenchymal stem cells in mice with lethal fulminant hepatic failure." Cell Transplant **22**(10): 1785-1799.

Large-scale production and noninvasive methods for harvesting mesenchymal stem cells (MSCs), particularly in elderly individuals, has prompted researchers to find new patient-specific sources for MSCs in regenerative medicine. This study aims to produce MSCs from human induced pluripotent stem cells (hiPSCs) and to evaluate their therapeutic effects in a CCl4-induced mouse model of fulminant hepatic failure (FHF). hiPSC-MSCs have shown MSC morphology, antigen profile and differentiation capabilities, and improved hepatic function in our model. hiPSC-MSC-transplanted animals provide significant benefit in terms of survival, serum LDH, total bilirubin, and lipid peroxidation. hiPSC-MSC therapy resulted in a one-third reduction of histologic activity index and a threefold increase in the number of proliferating hepatocytes. This was accompanied by a significant decrease in the expression levels of collagen type I, Mmp13, Mmp2, and Mmp9 genes and increase in Timp1 and Timp2 genes in transplanted groups. hiPSC-MSCs secreted hepatocyte growth factor (HGF) in vitro and also expressed HGF in evaluated liver sections. Similar results were observed with human bone marrow (hBM)-derived MSCs. In conclusion, our results have demonstrated that hiPSC-MSCs might be valuable appropriate alternatives for hBM-MSCs in FHF liver repair and support liver function by cell therapy with a large-scale production capacity, patient-specific nature, and no invasive MSC harvesting.

Motaln, H., et al. (2010). "Human mesenchymal stem cells and their use in cell-based therapies." Cancer **116**(11): 2519-2530.

The human population is increasingly facing various diseases, including types of cancer, that cannot be cured with conventional drugs. Advanced drug targeting of tumor cells is also often impossible when treating highly invasive and infiltrative tumors such as glioblastoma or pulmonary cancer, because of tumor cells' high migration and invasiveness. Pluripotent human mesenchymal stem cells (hMSCs) have been extensively studied, and strategies are being proposed for treating "incurable" cancers and injury/disease-affected organs. Because of their own intrinsic properties, involving homing and immunomodulatory potency, hMSCs could be used as an excellent cell/drug delivery vehicle in those cell-based therapies. Their unprecedented use has been shadowed, however, by their spontaneous transformation, which links them to cancer-initiating cells during tumor development. How malignant initiation proceeds in vivo, and what are the exact characteristics of the cancer-initiating cells, still remain to be investigated. In the present review, the authors summed up the most recent knowledge about hMSC characteristics, their malignant transformation, and outlined the possibilities of their safe use in novel cell-based therapies.

Mueller, T., et al. (2009). "Analysis of OCT4 expression in an extended panel of human tumor cell lines from multiple entities and in human mesenchymal stem cells." Cell Mol Life Sci **66**(3): 495-503.

OCT4 is considered a main regulator of embryonic stem cell pluripotency and self renewal capacity. It was shown that relevant OCT4 expression only occurs in cells of embryonic pluripotent nature. However, several recent publications claimed to have demonstrated OCT4 expression in human somatic tumor cells, human adult stem or progenitor cells and differentiated cells.We analysed 42 human tumor cell lines from 13 entities and human bone marrow-derived mesenchymal stem cells (MSC). To validate OCT4 expression we used germ cell tumor (GCT) cell lines, derived xenografts and GCT samples. Analysis by RT-

PCR, western blotting, immunocytochemistry and immunohistochemistry was performed. With exception of typical embryonal carcinoma cells, we did not observe reliable OCT4 expression in somatic tumor cell lines and MSC. We suggest that a high level of expression of the OCT4 protein together with its nuclear localization still remains a reliable and definitive feature of cells with embryonic pluripotent nature.

Mullick, M. and D. Sen (2018). "The Delta Opioid Peptide DADLE Represses Hypoxia-Reperfusion Mimicked Stress Mediated Apoptotic Cell Death in Human Mesenchymal Stem Cells in Part by Downregulating the Unfolded Protein Response and ROS along with Enhanced Anti-Inflammatory Effect." Stem Cell Rev **14**(4): 558-573.

Hypoxia-reperfusion (H/R) emblems a plethora of pathological conditions which is potent in contributing to the adversities encountered by human mesenchymal stem cells (hMSCs) in post-transplant microenvironment, resulting in transplant failure. D-Alanine 2, Leucine 5 Enkephaline (DADLE)-mediated delta opioid receptor (DOR) activation is well-known for its recuperative properties in different cell types like neuronal and cardiomyocytes. In the current study its effectiveness in assuaging hMSC mortality under H/R-like insult has been delineated. The CoCl2 mimicked H/R conditions in vitro was investigated upon DOR activation, mediated via DADLE. hMSCs loss of viability, reactive oxygen species (ROS) production, inflammatory responses and disconcerted unfolded protein response (UPR) were assessed using AnnexinV/PI flow cytometry, fluorescence imaging, mitochondrial complex 1 assay, quantitative PCR, immunoblot analysis and ELISA. H/R like stress induced apoptosis of hMSCs was significantly mitigated by DADLE via modulation of the apoptotic regulators (Bcl-2/Bax) along with significant curtailment of ROS and mitochondrial complex 1 activity. DADLE concomitantly repressed the misfolded protein aggregation, alongside the major UPR sensors: PERK/BiP/IRE-1alpha /ATF-6, evoked due to the H/R mimicked endoplasmic reticulum stress. Undermined phosphorylation of the Akt signalling pathway was observed, which concerted its effect onto regulating both the pro and anti-inflammatory cytokines, actuated as a response to the H/R-like insult. The effects of DADLE were subdued by naltrindole (specific DOR antagonist) reaffirming the involvement of DOR in the process. Taken together these results promulgate the role of DADLE-induced DOR activation on improved hMSC survival, which signifies the plausible implications of DOR-activation in cell-transplantation therapies and tissue engineering aspect.

Najimi, M., et al. (2017). "Human liver mesenchymal stem/progenitor cells inhibit hepatic stellate cell activation: in vitro and in vivo evaluation." Stem Cell Res Ther **8**(1): 131.

BACKGROUND: Progressive liver fibrosis leads to cirrhosis and end-stage liver disease. This disease is a consequence of strong interactions between matrix-producing hepatic stellate cells (HSCs) and resident and infiltrating immune cell populations. Accumulated experimental evidence supports the involvement of adult-derived human liver mesenchymal stem/progenitor cells (ADHLSCs) in liver regeneration. The aim of the present study was to evaluate the influence of ADHLSCs on HSCs, both in vitro and in vivo. METHODS: Activated human HSCs were co-cultured with ADHLSCs or ADHLSC-conditioned culture medium. The characteristics of the activated human HSCs were assessed by microscopy and biochemical assays, whereas proliferation was analyzed using flow cytometry and immunocytochemistry. The secretion profile of activated HSCs was evaluated by ELISA and Luminex. ADHLSCs were transplanted into a juvenile rat model of fibrosis established after co-administration of phenobarbital and CCl4. RESULTS: When co-cultured with ADHLSCs or conditioned medium, the proliferation of HSCs was inhibited, beginning at 24 h and for up to 7 days. The HSCs were blocked in G0/G1 phase, and showed decreased Ki-67 positivity. Pro-collagen I production was reduced, while secretion of HGF, IL-6, MMP1, and MMP2 was enhanced. Neutralization of HGF partially blocked the inhibitory effect of ADHLSCs on the proliferation and secretion profile of HSCs. Repeated intrahepatic transplantation of cryopreserved/thawed ADHLSCs without immunosuppression inhibited the expression of markers of liver fibrosis in 6 out of 11 rats, as compared to their expression in the vehicle-transplanted group. CONCLUSIONS: These data provide evidence for a direct inhibitory effect of ADHLSCs on activated HSCs, which supports their development for the treatment of liver fibrosis.

Nakahata, A. M., et al. (2010). "Human glioblastoma cells display mesenchymal stem cell features and form intracranial tumors in immunocompetent rats." J Stem Cells **5**(3): 103-111.

Isolation of highly tumorigenic stem-like cells from human glioblastoma specimens and cell lines has been focusing on their neural stem cells properties or capacity to efflux fluorescent dyes. Here, we report that, under standard culture conditions, human glioblastoma cells of the U87MG cell line display a predominant mesenchymal phenotype and share some of the in vitro properties of mesenchymal stem cells. Moreover, these cells were capable of forming tumors in

immunocompetent rats. Infiltrative intracranial tumors could be detected 15 to 30 days post-stereotaxic cell injection within the motor cortex. Tumors were comprised by pleomorphic and mitotically active cells and displayed necrotic and hemorrhagic foci, which are common features of human glioblastomas. This rather unexpected in vivo tumorigenesis in the absence of immune suppression more closely mimics the physiological milieu encountered by tumor cells and could be explored as a xenograft orthotopic model of human glioblastomas to address new therapeutic approaches, particularly those involving immune effector mechanisms.

Nakamura, K., et al. (2018). "Initial cell plating density affects properties of human primary synovial mesenchymal stem cells." J Orthop Res.

Synovial mesenchymal stem cells (MSCs) appear to be an attractive cell source in cartilage and meniscus regeneration because of their high proliferative and chondrogenic potentials. Two methods are used to culture synovial nucleated cells in the preparation of primary synovial MSCs. In one method, the cells are plated at low density to make cell colonies. In the other method, the cells are plated at high density. We investigated the effects of initial cell density on proliferation, surface markers, and multipotentiality, including chondrogenesis in primary synovial MSCs. Human synovium was obtained from the knee joints of patients with osteoarthritis after total knee arthroplasty. Immediately after enzyme digestion, the synovial nucleated cells were plated in densities of 10(3) , 10(4) , or 10(5) cells/60-cm(2) dish and cultured for 14 days. Proliferation, surface markers, chondrogenesis, adipogenesis, and calcification were examined in three populations. The cell colonies were distinct in the 10(3) cells/dish group, faint in the 10(4) cells/dish group, and obscure in the 10(5) cells/dish group. The total number of cells/dish was positively related to plating density, whereas the fold increase was negatively related to plating density (n = 13). Among 12 surface markers, a negative relation to plating density was distinct in CD105. The cartilage pellet weight was negatively related to the initial plating density. The oil red-o positive area and alizarin red positive area were positively related to the initial plating density. The initial cell plating density affected the properties of primary synovial MSCs. Synovial nucleated cells proliferated better when plated at low density, and the synovial MSCs obtained by this method contained a high chondrogenic potential. (c) 2018 The Authors. Journal of Orthopaedic Research(R) Published by Wiley Periodicals, Inc. J Orthop Res.

Nakayama, C., et al. (2018). "The development of induced pluripotent stem cell-derived mesenchymal stem/stromal cells from normal human and RDEB epidermal keratinocytes." J Dermatol Sci **91**(3): 301-310.

BACKGROUND: Epidermolysis bullosa (EB) is a group of hereditary disorders caused by mutations in the genes encoding structural molecules of the dermal-epidermal junction (DEJ). Cell-based therapies such as allogeneic mesenchymal stem/stromal cell (MSC) transplantation have recently been explored for severe EB types, such as recessive dystrophic EB (RDEB). However, hurdles exist in current MSC-based therapies, such as limited proliferation from a single cell source and limited cell survival due to potential allogenic rejection. OBJECTIVES: We aimed to develop MSCs from keratinocyte-derived induced pluripotent stem cells (iPSCs). METHODS: Keratinocyte-derived iPSCs (KC-iPSCs) of a healthy human and an RDEB patient were cultured with activin A, 6-bromoindirubin-3'-oxime and bone morphogenetic protein 4 to induce mesodermal lineage formation. These induced cells were subjected to immunohistochemical analysis, flow cytometric analysis and RNA microarray analysis in vitro, and were injected subcutaneously and intravenously to wounded immunodeficient mice to assess their wound-healing efficacy. RESULTS: After their induction, KC-iPSC-induced cells were found to be compatible with MSCs. Furthermore, with the subcutaneous and intravenous injection of the KC-iPSC-induced cells into wounded immunodeficient mice, human type VII collagen was detected at the DEJ of epithelized areas. CONCLUSIONS: We successfully established iPSC-derived MSCs from keratinocytes (KC-iPSC-MSCs) of a normal human and an RDEB patient. KC-iPSC-MSCs may have potential in therapies for RDEB.

Narang, H., et al. (2015). "Effect of proton and gamma irradiation on human lung carcinoma cells: Gene expression, cell cycle, cell death, epithelial-mesenchymal transition and cancer-stem cell trait as biological end points." Mutat Res **780**: 35-46.

Proton beam therapy is a cutting edge modality over conventional gamma radiotherapy because of its physical dose deposition advantage. However, not much is known about its biological effects vis-a-vis gamma irradiation. Here we investigated the effect of proton- and gamma-irradiation on cell cycle, death, epithelial-mesenchymal transition (EMT) and "stemness" in human non-small cell lung carcinoma cells (A549). Proton beam (3MeV) was two times more cytotoxic than gamma radiation and induced higher and longer cell cycle arrest. At equivalent doses, numbers of genes responsive to proton irradiation were ten times higher than those responsive to gamma irradiation. At equitoxic doses, the proton-irradiated cells had reduced cell adhesion

and migration ability as compared to the gamma-irradiated cells. It was also more effective in reducing population of Cancer Stem Cell (CSC) like cells as revealed by aldehyde dehydrogenase activity and surface phenotyping by CD44(+), a CSC marker. These results can have significant implications for proton therapy in the context of suppression of molecular and cellular processes that are fundamental to tumor expansion.

Narita, Y., et al. (2008). "Effects of transforming growth factor-beta 1 and ascorbic acid on differentiation of human bone-marrow-derived mesenchymal stem cells into smooth muscle cell lineage." Cell Tissue Res **333**(3): 449-459.

Bone-marrow-derived mesenchymal stem cells (MSCs) can differentiate into a variety of cell types including smooth muscle cells (SMCs). We have attempted to demonstrate that, following treatment with transforming growth factor-beta 1 (TGF-beta1) and ascorbic acid (AA), human bone-marrow-derived MSCs differentiate into the SMC lineage for use in tissue engineering. Quantitative polymerase chain reaction for SMC-specific gene (alpha smooth muscle actin, h1-calponin, and SM22alpha) expression was performed on MSCs, which were cultured with various concentrations of TGF-beta1 or AA. TGF-beta1 had a tendency to up-regulate the expression of SMC-specific genes in a dose-dependent manner. The expression of SM22alpha was significantly up-regulated by 30 microM AA. We also investigated the additive effect of TGF-beta1 and AA for differentiation into SMCs and compared this effect with that of other factors including platelet-derived growth factor BB (PDGF-BB). In addition to SMC-specific gene expression, SMC-specific proteins increased by two to four times when TGF-beta1 and AA were used together compared with their administration alone. PDGF did not increase the expression of SMC-specific markers. MSCs cultured with TGF-beta1 and AA did not differentiate into osteoblasts and adipocytes. These results suggest that a combination of TGF-beta1 and AA is useful for the differentiation of MSCs into SMCs for use in tissue engineering.

Nasef, A., et al. (2007). "Identification of IL-10 and TGF-beta transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells." Gene Expr **13**(4-5): 217-226.

Mesenchymal stem cells (MSC) inhibit the response of allogeneic T lymphocytes in culture. Because the mechanisms of this effect may differ according to the existence of cell contact, we investigated the differences in gene expression of inhibitory molecules during MSC-T lymphocyte coculture when cell contact does and does not occur. Human MSC and T lymphocytes were cultured together in standard and transwell cultures. MSC gene expression was analyzed by semiquantitative real-time RT-PCR. MSC elicited a high dose-dependent inhibition of T lymphocytes in cultures with cell contact, but inhibition occurred even without cell contact. In both cases, we observed significant upregulation of IDO, LIF, and HLA-G, along with downregulation of HGF and SDF1. In cultures with cell contact, IL-10 and TGF-beta transcripts were expressed in a significantly higher level than in cultures without this contact. Furthermore, in the latter, the increased inhibition of T-cell proliferation was positively correlated with IDO gene expression and negatively correlated with SDF1 gene expression. MSC appear to induce T-cell tolerance by two distinct mechanisms. The first of these, which does not require cell contact, induces expression of the tolerogenic genes IDO, LIF, and HLA-G. The second mechanism, which is contact dependent, modulates IL-10 and TGF-beta gene expression. These two mechanisms probably play separate roles in MSC-induced tolerance in allogeneic hematopoietic stem cell transplantation.

Nedopil, A., et al. (2010). "MR signal characteristics of viable and apoptotic human mesenchymal stem cells in matrix-associated stem cell implants for treatment of osteoarthritis." Invest Radiol **45**(10): 634-640.

OBJECTIVE: To compare magnetic resonance (MR) signal characteristics of contrast agent-labeled apoptotic and viable human mesenchymal stem cells (hMSCs) in matrix-associated stem cell implants. METHODS: hMSCs were labeled with Food and Drug Administration-approved ferumoxides nanoparticles. One group (A) remained untreated whereas a second group (B) underwent mitomycin C-induced apoptosis induction. Viability of group A and apoptosis of group B was confirmed by caspase-assays and terminal dUTP nick-end labeling (TUNEL) stains. Labeled viable hMSCs, unlabeled viable hMSCs, labeled apoptotic hMSCs, and unlabeled apoptotic hMSCs (n = 7 samples each) in an agarose scaffold were implanted into cartilage defects of porcine patellae specimens and underwent MR imaging at 7 T, using T1-weighted spin-echo sequences, T2-weighted spin-echo sequences, and T2*-weighted gradient-echo sequences. Signal-to-noise ratios (SNR) of the implants were calculated and compared between different experimental groups using linear mixed regression models. RESULTS: Ferumoxides-labeled hMSCs provided a strong negative T2 and T2*-enhancement. Corresponding SNR data of labeled hMSCs were significantly lower compared with unlabeled controls ($P < 0.05$). Apoptosis induction resulted in a significant signal decline of ferumoxides-labeled hMSC transplants on

short echo time T2-weighted spinecho sequences. SNR data of labeled apoptotic hMSCs were significantly lower compared with labeled viable hMSCs (P < 0.05). CONCLUSION: Apoptosis of transplanted ferumoxides-labeled stem cells in cartilage defects can be visualized noninvasively by a significant signal decline on T2-weighted MR images. The described MR signal characteristics may serve as a noninvasive outcome measure for the assessment of matrix-associated stem cell implants in clinical practice. Additional studies are needed to further enhance the observed differences between viable and apoptotic cells, for example, by further optimizing the applied MR pulse sequence parameters or intracellular contrast agent concentration.

Nejad, N. A., et al. (2015). "Male germ-like cell differentiation potential of human umbilical cord Wharton's jelly-derived mesenchymal stem cells in co-culture with human placenta cells in presence of BMP4 and retinoic acid." Iran J Basic Med Sci **18**(4): 325-333.

OBJECTIVES: Mesenchymal stem cells (MSCs) derived from Wharton's jelly (WJ-MSCs) are now much more appealing for cell-based infertility therapy. Hence, WJ-MSCs differentiation toward germ layer cells for cell therapy purposes is currently under intensive study. MATERIALS AND METHODS: MSCs were isolated from human Wharton's jelly and treated with BMP4, retinoic acid (RA) or co-cultured on human amniotic epithelial (HAE) and chorionic plate (HCP) placenta feeder cells. profile of POU5F1, Fragilis, Plzf, DDX4, Piwil2, Stra8, Dazl, beta1- and alpha6-integrins (ITBeta1, ITA6) genes expression as germ cell markers were analyzed using RT-PCR and real-time PCR. Immunocytochemistry of surface markers were conducted. RESULTS: After 3 weeks treatment with different reagents and co-culture system, morphology of WJ-MSCs changed to shiny clusters and germ cell specific markers in mRNA were up-regulated in both placental feeder + RA and BMP4 + RA. Induction of hWJ-MSCs with BMP4 in presence of RA resulted in significant up-regulation (P</=0.05) of all germ cell specific genes (c-Kit; 2.84+/-0.59, DDX4; 1.69+/-0.39, Piwil2; 1.14+/-0.21, Dazl; 0.65+/-0.25, alpha6 integrin; 1.26+/-0.53, beta1 integrins; 1.18+/-0.65) compared to control and placental feeder cells + RA. Our results indicated that HAE and HCP followed by RA treatment were involved in human germ cell development. CONCLUSION: We demonstrated that under the right conditions, hWJ-MSCs have the ability to differentiate to germ cells and this provides an excellent pattern to study infertility cause and treatment.

Neuss, S., et al. (2004). "Functional expression of HGF and HGF receptor/c-met in adult human mesenchymal stem cells suggests a role in cell mobilization, tissue repair, and wound healing." Stem Cells **22**(3): 405-414.

Human mesenchymal stem cells (hMSC) are adult stem cells with multipotent capacities. The ability of mesenchymal stem cells to differentiate into many cell types, as well as their high ex vivo expansion potential, makes these cells an attractive therapeutic tool for cell transplantation and tissue engineering. hMSC are thought to contribute to tissue regeneration, but the signals governing their mobilization, diapedesis into the bloodstream, and migration into the target tissue are largely unknown. Here we report that hepatocyte growth factor (HGF) and the cognate receptor HGFR/c-met are expressed in hMSC, on both the RNA and the protein levels. The expression of HGF was downregulated by transforming growth factor beta. HGF stimulated chemotactic migration but not proliferation of hMSC. Therefore the HGF/c-met signaling system may have an important role in hMSC recruitment sites of tissue regeneration. The controlled regulation of HGF/c-met expression may be beneficial in tissue engineering and cell therapy employing hMSC.

Nguyen, T. M., et al. (2013). "EphB and Ephrin-B interactions mediate human mesenchymal stem cell suppression of activated T-cells." Stem Cells Dev **22**(20): 2751-2764.

Mesenchymal stromal/stem cells (MSC) express the contact-dependent erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinase family and their cognate ephrin ligands, which are known to regulate thymocyte maturation and selection, T-cell transendothelial migration, activation, co-stimulation, and proliferation. However, the contribution of Eph/ephrin molecules in mediating human MSC suppression of activated T-cells remains to be determined. In the present study, we showed that EphB2 and ephrin-B2 are expressed by ex vivo expanded MSC, while the corresponding ligands, ephrin-B1 and EphB4, respectively, are highly expressed by T-cells. Initial studies demonstrated that EphB2-Fc and ephrin-B2-Fc molecules suppressed T-cell proliferation in allogeneic mixed lymphocyte reaction (MLR) assays compared with human IgG-treated controls. While the addition of a third-party MSC population demonstrated dramatic suppression of T-cell proliferation responses in the MLR, blocking the function of EphB2 or EphB4 receptors using inhibitor binding peptides significantly increased T-cell proliferation. Consistent with these observations, shRNA EphB2 or ephrin-B2 knockdown expression in MSC reduced their ability to inhibit T-cell proliferation. Importantly, the expression of immunosuppressive factors, indoleamine 2, 3-dioxygenase, transforming growth factor-beta1, and inducible nitric oxide synthase expressed by MSC, was up-regulated after

stimulation with EphB4 and ephrin-B1 in the presence of interferon (IFN)-gamma, compared with untreated controls. Conversely, key factors involved in T-cell activation and proliferation, such as interleukin (IL)-2, IFN-gamma, tumor necrosis factor-alpha, and IL-17, were down-regulated by T-cells treated with EphB2 or ephrin-B2 compared with untreated controls. Studies utilizing signaling inhibitors revealed that inhibition of T-cell proliferation is partly mediated through EphB2-induced ephrin-B1 reverse signaling or ephrin-B2-mediated EphB4 forward signaling by activating Src, PI3Kinase, Abl, and JNK kinase pathways, activated by tyrosine phosphorylation. Taken together, these observations suggest that EphB/ephrin-B interactions play an important role in mediating human MSC inhibition of activated T cells.

Nieto-Miguel, T., et al. (2013). "In vitro simulation of corneal epithelium microenvironment induces a corneal epithelial-like cell phenotype from human adipose tissue mesenchymal stem cells." Curr Eye Res **38**(9): 933-944.

PURPOSE: Transplantation of autologous corneal stem cells in not possible in cases of bilateral limbal stem cell deficiency (LSCD). To restore the ocular surface in these patients, an autologous extraocular source of stem cells is desirable to avoid dependence on deceased donor tissue and host immunosuppression of allogenic transplants. While bone marrow-derived mesenchymal stem cells (MSCs) can acquire certain characteristics of corneal epithelial cells, subcutaneous adipose tissue (AT) is more readily available and accessible. The aim of this study was to determine if extraocular human AT-derived MSCs (hAT-MSCs) can acquire in vitro some features of corneal epithelial-like cells. METHODS: hAT-MSCs were isolated from human lipoaspirates and expanded up to 3-4 passages. We studied the immunophenotype of MSCs and demonstrated its multipotent capacity to differentiate toward osteoblasts, adipocytes and chondrocytes. To test the capacity of differentiation of hAT-MSCs toward corneal epithelial-like cells, hAT-MSCs were cultured on substrata of plastic or collagen IV. We used basal culture medium (BM), BM conditioned with human corneal epithelial cells (HCEcBM) and BM conditioned with limbal fibroblasts (LFcBM). RESULTS: The hAT-MSCs incubated for 15 days with HCEcBM acquired more polygonal and complex morphology as evaluated by phase-contrast microscopy and flow cytometry. Additionally, the expression of transforming growth factor-beta receptor CD105 and corneal epithelial marker CK12 got increased as evaluated by flow cytometry, real-time reverse-transcription polymerase chain reaction, western blot and immunostaining. These changes were absent in hAT-MSCs incubated with unconditioned BM or with LFcBM. CONCLUSIONS: Corneal epithelial-like cells can be induced from extraocular hAT-MSCs by subjecting them to an in vitro microenvironment containing conditioning signals derived from differentiated human corneal epithelial cells. Our results suggest that hAT-MSCs could provide a novel source of stem cells that hold the potential to restore sight lost in patients suffering from bilateral ocular surface failure due to LSCD.

Nikolaev, N. I., et al. (2014). "Changes in the stiffness of human mesenchymal stem cells with the progress of cell death as measured by atomic force microscopy." J Biomech **47**(3): 625-630.

This note reports observations of the change of stiffness of human mesenchymal stem cells (hMSCs) with the progress of cell death as measured by AFM. hMSC with impaired membrane, dead and viable cells were labelled with Annexin V and Propidium Iodide after 24h cold storage, followed by AFM measurement and Young's modulus of cells was derived. Viable hMSCs have a Young's modulus (E) in the range of 0.81-1.13kPa and consistent measurement was observed when different measurement locations were chosen. E of cells with partially impaired membrane was 0.69+/-0.17kPa or in the range of 2.04-4.74kPa, depending upon the measurement locations. With the loss of membrane integrity, though there was no variation on measured E between different locations, a mixed picture of cell stiffness was observed as indicated by cells with E as low as 0.09+/-0.03kPa, in a mid-range of 4.62+/-0.67kPa, and the highest of up to 48.98+/-19.80kPa. With the progress of cell death, the highest stiffness was noticed for cells showing a more granular appearance; also the lowest stiffness for cells with vacuole appearance. Findings from this study indicate that cell stiffness is significantly altered with the progress of cell death.

Noh, Y. K., et al. (2016). "Polymer mesh scaffold combined with cell-derived ECM for osteogenesis of human mesenchymal stem cells." Biomater Res **20**: 6.

BACKGROUND: Tissue-engineered scaffold should mimic the structure and biological function of the extracellular matrix and have mechanically supportive properties for tissue regeneration. In this study, we utilized a PLGA/PLA mesh scaffold, coated with cell-derived extracellular matrix (CDM) and assessed its potential as an osteogenic microenvironment for human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs). CDM was obtained by decellularization of in vitro-cultured type I collagen overexpressing (Col I -293 T-DK) cells. Test groups are mesh itself (control), fibronectin-coated (FN-mesh), and CDM-coated mesh scaffold

(CDM-mesh). CDM was then solubilized and used for scaffold coating. RESULTS: CDM was successfully collected and applied to mesh scaffolds. The presence of CDM was confirmed via SEM and FN immunofluorescence. After then, UCB-MSCs were seeded into the scaffolds and subjected to the induction of osteogenic differentiation for 21 days in vitro. We found that the seeded cells were viable and have better proliferation activity on CDM-mesh scaffold. In addition, when osteogenic differentiation of UCB-MSCs was examined for up to 21 days, alkaline phosphatase (ALP) activity and osteogenic marker (COL I, ALP, osteocalcin, bone sialoprotein) expression were significantly improved with UCB-MSCs when cultured in the CDM-mesh scaffold compared to the control and FN-mesh. CONCLUSION: Polymer mesh scaffold incorporated with CDM can provide UCB-MSCs with a better microenvironment for osteogenesis in vitro.

Ozdemir, A. T., et al. (2016). "The paracrine immunomodulatory interactions between the human dental pulp derived mesenchymal stem cells and CD4 T cell subsets." Cell Immunol **310**: 108-115.

Mesenchymal stem cells (MSCs) have strong immunomodulatory properties, however these properties may show some differences according to the tissue type of their isolate. In this study we investigated the paracrine interactions between human DP derived MSCs (hDP-MSCs) and the CD4(+) T helper cell subsets to establish their immunomodulatory mechanisms. We found that the CD4(+)-Tbet(+) (Th1) and CD4(+)-Gata3(+) (Th2) cells were suppressed by the hDP-MSCs, but the CD4(+)-Stat3(+) (Th17) and CD4(+)-CD25(+)-FoxP3(+) (Treg) cells were stimulated. The expressions of T cell specific cytokines interferon gamma (IFN-g), interleukin (IL)-4 and IL-17a decreased, but IL-10 and transforming growth factor beta-1 (TGF-b1) increased with the hDP-MSCs. The expressions of indoleamine-pyrrole 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), soluble human leukocyte antigen G (sHLA-G) derived from hDP-MSCs slightly increased, but hepatocyte growth factor (HGF) significantly increased in the co-culture groups. According to our findings, the hDP-MSCs can suppress the Th1 and Th2 subsets but stimulate the Th17 and Treg subsets. The Stat3 expression of Th17 cells may have been stimulated by the HGF, and thus the pro-inflammatory Th17 cells may have altered into the immunosuppressive regulatory Th17 cells. Further prospective studies are needed to confirm our findings.

Ozeki, N., et al. (2016). "Polyphosphate-induced matrix metalloproteinase-13 is required for osteoblast-like cell differentiation in human adipose tissue derived mesenchymal stem cells." Biosci Trends **10**(5): 365-371.

Inorganic polyphosphate [Poly(P)] induces differentiation of osteoblastic cells. In this study, matrix metalloproteinase (MMP)-13 small interfering RNA (siRNA) was transfected into human adipose tissue-derived mesenchymal stem cells (hAT-MSC) to investigate whether MMP-13 activity induced by Poly(P) is associated with osteogenic differentiation. Real-time quantitative polymerase chain reaction, Western blotting, and an MMP-13 activity assay were used in this study. Poly(P) enhanced expression of mature osteoblast markers, such as osteocalcin (BGLAP) and osteopontin (SPP1), osterix (OSX), and bone sialoprotein (BSP), and increased alkaline phosphatase (ALP) activity and calcification capacity in hAT-MSCs. These cells also developed an osteogenic phenotype with increased expression of Poly(P)-induced expression of MMP-13 mRNA and protein, and increased MMP-13 activity. MMP-13 siRNA potently suppressed the expression of osteogenic biomarkers BGLAP, SPP1, OSX, BSP, and ALP, and blocked osteogenic calcification. Taken together, Poly(P)-induced MMP-13 regulates differentiation of osteogenic cells from hAT-MSCs.

Pan, H. C. (2015). "Retraction: Enhancement of regeneration with glia cell line-derived neurotrophic factor-transduced human amniotic fluid mesenchymal stem cells after sciatic nerve crush injury." J Neurosurg **123**(6): 1606.

Papadimitriou, N., et al. (2014). "Cell viability and chondrogenic differentiation capability of human mesenchymal stem cells after iron labeling with iron sucrose." Stem Cells Dev **23**(21): 2568-2580.

For evaluation of cell therapy strategies using human mesenchymal stem cells (hMSCs), it is important to be able to trace transplanted cells and their distribution in tissues, for example, cartilage, over time. The aim of the study was to determine effects on cell viability, traceability, and chondrogenic differentiation of hMSCs after iron labeling with iron sucrose. hMSCs were collected (seven donors, 13-57 years) from patients undergoing spinal surgery. Two subsets of experiments were performed. (1) Iron labeling of hMSCs: 1 mg/mL of Venofer((R)) (iron sucrose) was added (16 h) to cultures. hMSCs were examined for uptake of iron sucrose (Prussian blue staining) and cell viability (flow cytometry). (2) Iron-labeled hMSCs (passage 4) (n=4, pellet mass), 200,000 cells/tube, were cultured (DMEM-HG) with 10 ng/mL TGFbeta and compared with controls (from each donor). The pellets were harvested at days 7, 14, and 28. Real-time PCR, IHC, and histology were used to evaluate SOX9, ACAN, C6S, and COL2A1 expression. Mean number

of cells containing iron deposits was 98.1% and mean cell viability was 92.7% (no significant difference compared with unlabeled control cells). Pellets containing iron-labeled cells expressed COL2A1 on protein level (all time points), in similar levels as controls, and glycosaminoglycan accumulation was observed in iron-labeled pellets (day 14 or day 28). Results were supported by the expression of chondrogenic genes SOX9, ACAN, and COL2A1. The results in vitro indicate that iron sucrose can be used as a cell tracer for evaluation of cellular distribution in vivo after transplantation of MSCs and thus contribute with important knowledge when exploring new treatment strategies for degenerated cartilaginous tissues.

Park, H. E., et al. (2011). "Real-time monitoring of neural differentiation of human mesenchymal stem cells by electric cell-substrate impedance sensing." J Biomed Biotechnol **2011**: 485173.

Stem cells are useful for cell replacement therapy. Stem cell differentiation must be monitored thoroughly and precisely prior to transplantation. In this study we evaluated the usefulness of electric cell-substrate impedance sensing (ECIS) for in vitro real-time monitoring of neural differentiation of human mesenchymal stem cells (hMSCs). We cultured hMSCs in neural differentiation media (NDM) for 6 days and examined the time-course of impedance changes with an ECIS array. We also monitored the expression of markers for neural differentiation, total cell count, and cell cycle profiles. Cellular expression of neuron and oligodendrocyte markers increased. The resistance value of cells cultured in NDM was automatically measured in real-time and found to increase much more slowly over time compared to cells cultured in non-differentiation media. The relatively slow resistance changes observed in differentiating MSCs were determined to be due to their lower growth capacity achieved by induction of cell cycle arrest in G0/G1. Overall results suggest that the relatively slow change in resistance values measured by ECIS method can be used as a parameter for slowly growing neural-differentiating cells. However, to enhance the competence of ECIS for in vitro real-time monitoring of neural differentiation of MSCs, more elaborate studies are needed.

Perez-Ilzarbe, M., et al. (2009). "Comparison of ex vivo expansion culture conditions of mesenchymal stem cells for human cell therapy." Transfusion **49**(9): 1901-1910.

BACKGROUND: Mesenchymal stem cells (MSCs) are multipotent stem cells. Based on their properties, several clinical trials have been designed to explore their potential therapeutic effect. Fetal calf serum (FCS, commonly used for in vitro expansion) is an undesirable source of xenogeneic antigens and bears the risk of transmitting contaminations. As an alternative for FCS, platelet lysate (PL) and both autologous and allogeneic human serum have been proposed. The aim of this study is to compare the culture of bone marrow (BM)-derived MSCs in the presence of different serum supplements to determine the effect on cell growth, differentiation potential, and immunologic function. STUDY DESIGN AND METHODS: MSCs from BM of healthy volunteer donors were grown in the presence of 10% FCS supplemented with 1 ng/mL basic fibroblast growth factor (bFGF), 10% human serum supplemented with 1 ng/mL bFGF, 5% PL, and PL 5% supplemented with 1 ng/mL bFGF (PL plus bFGF). RESULTS: MSCs that expanded in either medium showed a comparable morphology, phenotype, and proliferative and differentiation capacity. While the presence of MSCs in vitro significantly decreased CD3/CD28-mediated T-cell activation, this effect was significantly higher in MSCs cultured with human serum. Production of interferon-gamma was inhibited by cocultured media with MSCs while MSCs also induced a significant inhibition of cell cycle in T cells. DISCUSSION: In conclusion, PL or autologous serum could offer an alternative to the use of FCS in MSC expansion for clinical use maintaining the same growing potential, phenotype, immunomodulatory properties, and differentiation potential.

Periasamy, V. S., et al. (2016). "Fe3 O4 nanoparticle redox system modulation via cell-cycle progression and gene expression in human mesenchymal stem cells." Environ Toxicol **31**(8): 901-912.

The use of engineered nanoparticles (NPs) across multiple fields and applications has rapidly increased over the last decade owing to their unusual properties. However, there is an increased need in understanding their toxicological effect on human health. Particularly, iron oxide (Fe3 O4) have been used in various sectors, including biomedical, food, and agriculture, but the current understanding of their impact on human health is inadequate. In this investigation, we assessed the toxic effect of Fe3 O4 NPs on human mesenchymal stem cells (hMSCs) adopting cell viability, cellular morphological changes, mitochondrial transmembrane potential, and cell-cycle progression assessment methodologies. Furthermore, the expression of oxidative stress, cell death, and cell-cycle regulatory genes was assessed using quantitative polymerase chain reaction. The Fe3 O4 NPs induced cytotoxicity and nuclear morphological changes in hMSCs by dose and time exposure. Cell-cycle analysis indicated that Fe3 O4 NPs altered the cell-cycle progression through a decrease in the proportion of

cells in the G0 -G1 phase. The hMSC mitochondrial membrane potential loss increased with an increase in the concentration of Fe3 O4 NPs exposure. The observed expression levels of the CYP1A, TNF3, TNFSF10, E2F1, and CCNC genes were significantly upregulated in hMSCs in response to Fe3 O4 NPs exposure. Our findings suggest that Fe3 O4 NPs caused metabolic stress through altered cell cycle, oxidative stress, and cell death regulatory gene expression in hMSCs. The results of this investigation revealed that Fe3 O4 NPs exhibited moderate toxicity on hMSCs and that Fe3 O4 NPs may have biomedical applications at low concentrations. (c) 2014 Wiley Periodicals, Inc. Environ Toxicol 31: 901-912, 2016.

Periasamy, V. S., et al. (2016). "Aluminum oxide nanoparticles alter cell cycle progression through CCND1 and EGR1 gene expression in human mesenchymal stem cells." Biotechnol Appl Biochem **63**(3): 320-327.

Aluminum oxide nanoparticles (Al2 O3 -NPs) are important ceramic materials that have been used in a variety of commercial and industrial applications. However, the impact of acute and chronic exposure to Al2 O3 -NPs on the environment and on human health has not been well studied. In this investigation, we evaluated the cytotoxic effects of Al2 O3 -NPs on human mesenchymal stem cells (hMSCs) by using a cell viability assay and observing cellular morphological changes, analyzing cell cycle progression, and monitoring the expression of cell cycle response genes (PCNA, EGR1, E2F1, CCND1, CCNC, CCNG1, and CYCD3). The Al2 O3 -NPs reduced hMSC viability in a dose- and time-dependent manner. Nuclear condensation and fragmentation, chromosomal DNA fragmentation, and cytoplasmic vacuolization were observed in Al2 O3 -NP-exposed cells. The nuclear morphological changes indicated that Al2 O3 -NPs alter cell cycle progression and gene expression. The cell cycle distribution revealed that Al2 O3 -NPs cause cell cycle arrest in the sub-G0-G1 phase, and this is associated with a reduction in the cell population in the G2/M and G0/G1 phases. Moreover, Al2 O3 -NPs induced the upregulation of cell cycle response genes, including EGR1, E2F1, and CCND1. Our results suggested that exposure to Al2 O3 -NPs could cause acute cytotoxic effects in hMSCs through cell cycle regulatory genes.

Pinho, S., et al. (2013). "PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion." J Exp Med **210**(7): 1351-1367.

The intermediate filament protein Nestin labels populations of stem/progenitor cells, including self-renewing mesenchymal stem cells (MSCs), a major constituent of the hematopoietic stem cell (HSC) niche. However, the intracellular location of Nestin prevents its use for prospective live cell isolation. Hence it is important to find surface markers specific for Nestin(+) cells. In this study, we show that the expression of PDGFRalpha and CD51 among CD45(-) Ter119(-) CD31(-) mouse bone marrow (BM) stromal cells characterizes a large fraction of Nestin(+) cells, containing most fibroblastic CFUs, mesenspheres, and self-renewal capacity after transplantation. The PDGFRalpha(+) CD51 (+)subset of Nestin(+) cells is also enriched in major HSC maintenance genes, supporting the notion that niche activity co-segregates with MSC activity. Furthermore, we show that PDGFRalpha(+) CD51(+) cells in the human fetal BM represent a small subset of CD146(+) cells expressing Nestin and enriched for MSC and HSC niche activities. Importantly, cultured human PDGFRalpha(+) CD51(+) nonadherent mesenspheres can significantly expand multipotent hematopoietic progenitors able to engraft immunodeficient mice. These results thus indicate that the HSC niche is conserved between the murine and human species and suggest that highly purified nonadherent cultures of niche cells may represent a useful novel technology to culture human hematopoietic stem and progenitor cells.

Pisati, F., et al. (2007). "Induction of neurotrophin expression via human adult mesenchymal stem cells: implication for cell therapy in neurodegenerative diseases." Cell Transplant **16**(1): 41-55.

In animal models of neurological disorders for cerebral ischemia, Parkinson's disease, and spinal cord lesions, transplantation of mesenchymal stem cells (MSCs) has been reported to improve functional outcome. Three mechanisms have been suggested for the effects of the MSCs: transdifferentiation of the grafted cells with replacement of degenerating neural cells, cell fusion, and neuroprotection of the dying cells. Here we demonstrate that a restricted number of cells with differentiated astroglial features can be obtained from human adult MSCs (hMSCs) both in vitro using different induction protocols and in vivo after transplantation into the developing mouse brain. We then examined the in vitro differentiation capacity of the hMSCs in coculture with slices of neonatal brain cortex. In this condition the hMSCs did not show any neuronal transdifferentiation but expressed neurotrophin low-affinity (NGFR(p75)) and high-affinity (trkC) receptors and released nerve growth factor (NGF) and neurotrophin-3 (NT-3). The same neurotrophin's expression was demonstrated 45 days after the intracerebral transplantation of hMSCs into nude mice with surviving astroglial cells. These data further confirm the limited capability of adult hMSC to differentiate into neurons whereas they differentiated in

astroglial cells. Moreover, the secretion of neurotrophic factors combined with activation of the specific receptors of transplanted hMSCs demonstrated an alternative mechanism for neuroprotection of degenerating neurons. hMSCs are further defined in their transplantation potential for treating neurological disorders.

Polacek, M., et al. (2011). "The secretory profiles of cultured human articular chondrocytes and mesenchymal stem cells: implications for autologous cell transplantation strategies." Cell Transplant **20**(9): 1381-1393.

This study was undertaken to compare the phenotype of human articular chondrocytes (ACs) and bone marrow-derived mesenchymal stem cells (MSCs) after cell expansion by studying the spectrum of proteins secreted by cells into the culture medium. ACs and MSCs were expanded in monolayer cultures for some weeks, as done in standard cell transplantation procedures. Initially, the expression of cartilage signature genes was compared by real-time PCR. Metabolic labeling of proteins (SILAC) in combination with mass spectrometry (LC/MS-MS) was applied to investigate differences in released proteins. In addition, multiplex assays were carried out to quantify the amounts of several matrix metalloproteases (MMPs) and their natural inhibitors (TIMPs). Expanded chondrocytes showed a slightly higher expression of cartilage-specific genes than MSCs, whereas the overall spectra of released proteins were very similar for the two cell types. In qualitative terms MSCs seemed to secrete similar number of extracellular matrix proteins (43% vs. 45% of total proteins found) and catabolic agents (9% vs. 10%), and higher number of anabolic agents (12 % vs. 7%) compared to ACs. Some matrix-regulatory agents such as serpins, BMP-1, and galectins were detected only in MSC supernatants. Quantitative analyses of MMPs and TIMPs revealed significantly higher levels of MMP-1, MMP-2, MMP-3, and MMP-7 in the medium of ACs. Our data show that after the expansion phase, both ACs and MSCs express a dedifferentiated phenotype, resembling each other. ACs hold a phenotype closer to native cartilage at the gene expression level, whereas MSCs show a more anabolic profile by looking at the released proteins pattern. Our data together with the inherent capability of MSCs to maintain their differentiation potential for longer cultivation periods would favor the use of these cells for cartilage reconstruction.

Polzer, H., et al. (2010). "Quantification of fluorescence intensity of labeled human mesenchymal stem cells and cell counting of unlabeled cells in phase-contrast imaging: an open-source-based algorithm." Tissue Eng Part C Methods **16**(6): 1277-1285.

Assessment of cell fate is indispensable to evaluate cell-based therapies in regenerative medicine. Therefore, a widely used technique is fluorescence labeling. A major problem still is the standardized, noninvasive, and reliable quantification of fluorescence intensity of adherent cell populations on single-cell level, since total fluorescence intensity must be correlated to the cell number. Consequently, the aim of the present study was to produce and validate an open-source-based algorithm, capable of measuring the total fluorescence intensity of cell populations and assessing the total cell number in phase-contrast images. To verify the algorithms' capacity to assess fluorescence intensity, human mesenchymal stem cells were transduced to stably express enhanced green fluorescent protein and results produced by the algorithm were compared to flow cytometry analysis. No significant differences could be observed at any time ($p >/= 0.443$). For validation of the algorithm for cell counting in phase-contrast images, adherent human mesenchymal stem cells were manually counted and compared to results produced by the algorithm (correlation coefficient [CC] $r = 0.975$), nuclei staining (CC $r = 0.997$), and hemocytometer (CC $r = 0.629$). We conclude that applying the developed algorithm in routine practice allows robust, fast, and reproducible assessment of fluorescence intensity and cell numbers in simple large-scale microscopy. The method is easy to perform and open source based.

Portas, M., et al. (2016). "Use of Human Cadaveric Mesenchymal Stem Cells for Cell Therapy of a Chronic Radiation-Induced Skin Lesion: A Case Report." Radiat Prot Dosimetry **171**(1): 99-106.

Acute and late radiation-induced injury on skin and subcutaneous tissues are associated with substantial morbidity in radiation therapy, interventional procedures and also are of concern in the context of nuclear or radiological accidents. Pathogenesis is initiated by depletion of acutely responding epithelial tissues and damage to vascular endothelial microvessels. Efforts for medical management of severe radiation-induced lesions have been made. Nevertheless, the development of strategies to promote wound healing, including stem cell therapy, is required. From 1997 to 2014, over 248 patients were referred to the Radiopathology Committee of Hospital de Quemados del Gobierno de la Ciudad de Buenos Aires (Burns Hospital) for the diagnosis and therapy of radiation-induced localized lesions. As part of the strategies for the management of severe cases, there is an ongoing research and development protocol on 'Translational Clinical Trial phases I/II to evaluate the safety and efficacy of adult mesenchymal stem cells from bone marrow for the treatment of large burns and radiological lesions'. The object of this work was to

describe the actions carried out by the Radiopathology Committee of the Burns Hospital in a chronic case with more than 30 years of evolution without positive response to conventional treatments. The approach involved the evaluation of the tissular compromise of the lesion, the prognosis and the personalized treatment, including regenerative therapy.

Potapova, I. A., et al. (2010). "Von willebrand factor increases endothelial cell adhesiveness for human mesenchymal stem cells by activating p38 mitogen-activated protein kinase." Stem Cell Res Ther **1**(5): 35.

INTRODUCTION: Delivered systemically or natively circulating mesenchymal stem cells accumulate in injured tissues. During homing mesenchymal stem cells adhere to endothelial cells and infiltrate underlying tissue. Previously we have shown that adhesiveness of endothelial cells for mesenchymal stem cells correlates with the inhibition of mitochondrial function of endothelial cells and secretion of von Willebrand factor. We hypothesized that von Willebrand factor is an auto/paracrine regulator of endothelial cell adhesiveness and studied the effect of von Willebrand factor on adhesion of mesenchymal stem cells to endothelial cells. METHODS: We used Affymetrix DNA microarrays, human protein phospho-MAPK array, Western blot, cell-based ELISA and flow cytometry analysis to study the activation of endothelial cells by von Willebrand factor. Cell adhesion assay and protein kinase inhibitors were used to evaluate the role of mitogen-activated protein kinases in the regulation of endothelial cell adhesiveness for mesenchymal stem cell. RESULTS: Treatment of endothelial cells with von Willebrand factor stimulated the mesenchymal stem cell adhesion in a time- and concentration-dependent manner. Mesenchymal stem cells did not adhere to immobilized von Willebrand factor and did not express receptors for von Willebrand factor suggesting that the stimulation of the mesenchymal stem cell adhesion is a result of endothelial cell activation with von Willebrand factor. Treatment of endothelial cells with von Willebrand factor activated ERK-1,2 and p38 MAPK without an effect on gene or cell surface expression of E-selectin, P-selectin, VCAM1 and ICAM1. Inhibition of p38 MAPK, but not ERK-1,2, in endothelial cells completely abrogated the stimulation of the mesenchymal stem cell adhesion by von Willebrand factor. CONCLUSIONS: Von Willebrand factor is an auto/paracrine regulator of endothelial cells. Activation of p38 MAPK in endothelial cells by von Willebrand factor is responsible for the regulation of endothelial cell adhesiveness for mesenchymal stem cells.

Potdar, P. D. and S. B. D'Souza (2010). "Ascorbic acid induces in vitro proliferation of human subcutaneous adipose tissue derived mesenchymal stem cells with upregulation of embryonic stem cell pluripotency markers Oct4 and SOX 2." Hum Cell **23**(4): 152-155.

Mesenchymal stem cells (MSCs) have immense therapeutic potential because of their ability to self-renew and differentiate into various connective tissue lineages. The in vitro proliferation and expansion of these cells is necessary for their use in stem cell therapy. Recently our group has developed and characterized mesenchymal stem cells from subcutaneous and visceral adipose tissue. We observed that these cells show a slower growth rate at higher passages and therefore decided to develop a supplemented medium, which will induce proliferation. Choi et al. have recently shown that the use of ascorbic acid enhances the proliferation of bone marrow derived MSCs. We therefore studied the effect of ascorbic acid on the proliferation of MSCs and characterized their phenotypes using stem cell specific molecular markers. It was observed that the use of 250 muM ascorbic acid promoted the significant growth of MSCs without loss of phenotype and differentiation potential. There was no considerable change in gene expression of cell surface markers CD105, CD13, Nanog, leukemia inhibitory factor (LIF) and Keratin 18. Moreover, the MSCs maintained in the medium supplemented with ascorbic acid for a period of 4 weeks showed increase in pluripotency markers Oct4 and SOX 2. Also cells in the experimental group retained the typical spindle shaped morphology. Thus, this study emphasizes the development of suitable growth medium for expansion of MSCs and maintenance of their undifferentiated state for further therapeutic use.

Prieto, P., et al. (2016). "Cell Expansion-Dependent Inflammatory and Metabolic Profile of Human Bone Marrow Mesenchymal Stem Cells." Front Physiol **7**: 548.

Stem cell therapy has emerged as a promising new area in regenerative medicine allowing the recovery of viable tissues. Among the many sources of adult stem cells, bone marrow-derived are easy to expand in culture via plastic adherence and their multipotentiality for differentiation make them ideal for clinical applications. Interestingly, several studies have indicated that MSCs expansion in vitro may be limited mainly due to "cell aging" related to the number of cell divisions in culture. We have determined that MSCs exhibit a progressive decline across successive passages in the expression of stem cell markers, in plasticity and in the inflammatory response, presenting low immunogenicity. We have exposed human MSCs after several passages to TLRs ligands and analyzed their inflammatory response. These cells responded to

pro-inflammatory stimuli (i.e., NOS-2 expression) and to anti-inflammatory cytokines (i.e., HO1 and Arg1) until two expansions, rapidly declining upon subculture. Moreover, in the first passages, MSCs were capable to release IL1beta, IL6, and IL8, as well as to produce active MMPs allowing them to migrate. Interestingly enough, after two passages, anaerobic glycolysis was enhanced releasing high levels of lactate to the extracellular medium. All these results may have important implications for the safety and efficacy of MSCs-based cell therapies.

Pustlauk, W., et al. (2017). "Modulation of chondrogenic differentiation of human mesenchymal stem cells in jellyfish collagen scaffolds by cell density and culture medium." J Tissue Eng Regen Med **11**(6): 1710-1722.

Studies on tissue-engineering approaches for the regeneration of traumatized cartilage focus increasingly on multipotent human mesenchymal stem cells (hMSCs) as an alternative to autologous chondrocytes. The present study applied porous scaffolds made of collagen from the jellyfish Rhopilema esculentum for the in vitro chondrogenic differentiation of hMSCs. Culture conditions in those scaffolds differ from conditions in high-density pellet cultures, making a re-examination of these data necessary. We systematically investigated the influence of seeding density, basic culture media [Dulbecco's modified Eagle's medium (DMEM), alpha-minimum essential medium (alpha-MEM)] with varying glucose content and supplementation with fetal calf serum (FCS) or bovine serum albumin (BSA) on the chondrogenic differentiation of hMSCs. Gene expression analyses of selected markers for chondrogenic differentiation and hypertrophic development were conducted. Furthermore, the production of cartilage extracellular matrix (ECM) was analysed by quantification of sulphated glycosaminoglycan and collagen type II contents. The strongest upregulation of chondrogenic markers, along with the highest ECM deposition was observed in scaffolds seeded with 2.4 x 10(6) cells/cm(3) after cultivation in high-glucose DMEM and 0.125% BSA. Lower seeding densities compared to high-density pellet cultures were sufficient to induce in vitro chondrogenic differentiation of hMSCs in collagen scaffolds, which reduces the amount of cells required for the seeding of scaffolds and thus the monolayer expansion period. Furthermore, examination of the impact of FCS and alpha-MEM on chondrogenic MSC differentiation is an important prerequisite for the development of an osteochondral medium for simultaneous osteogenic and chondrogenic differentiation in biphasic scaffolds for osteochondral tissue regeneration. Copyright (c) 2015 John Wiley & Sons, Ltd.

Qi, X., et al. (2016). "Exosomes Secreted by Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Repair Critical-Sized Bone Defects through Enhanced Angiogenesis and Osteogenesis in Osteoporotic Rats." Int J Biol Sci **12**(7): 836-849.

Bone defects caused by trauma, severe infection, tumor resection and skeletal abnormalities are common osteoporotic conditions and major challenges in orthopedic surgery, and there is still no effective solution to this problem. Consequently, new treatments are needed to develop regeneration procedures without side effects. Exosomes secreted by mesenchymal stem cells (MSCs) derived from human induced pluripotent stem cells (hiPSCs, hiPSC-MSC-Exos) incorporate the advantages of both MSCs and iPSCs with no immunogenicity. However, there are no reports on the application of hiPSC-MSC-Exos to enhance angiogenesis and osteogenesis under osteoporotic conditions. HiPSC-MSC-Exos were isolated and identified before use. The effect of hiPSC-MSC-Exos on the proliferation and osteogenic differentiation of bone marrow MSCs derived from ovariectomized (OVX) rats (rBMSCs-OVX) in vitro were investigated. In vivo, hiPSC-MSC-Exos were implanted into critical size bone defects in ovariectomized rats, and bone regeneration and angiogenesis were examined by microcomputed tomography (micro-CT), sequential fluorescent labeling analysis, microfil perfusion and histological and immunohistochemical analysis. The results in vitro showed that hiPSC-MSC-Exos enhanced cell proliferation and alkaline phosphatase (ALP) activity, and up-regulated mRNA and protein expression of osteoblast-related genes in rBMSCs-OVX. In vivo experiments revealed that hiPSC-MSC-Exos dramatically stimulated bone regeneration and angiogenesis in critical-sized calvarial defects in ovariectomized rats. The effect of hiPSC-MSC-Exos increased with increasing concentration. In this study, we showed that hiPSC-MSC-Exos effectively stimulate the proliferation and osteogenic differentiation of rBMSCs-OVX, with the effect increasing with increasing exosome concentration. Further analysis demonstrated that the application of hiPSC-MSC-Exos+beta-TCP scaffolds promoted bone regeneration in critical-sized calvarial defects by enhancing angiogenesis and osteogenesis in an ovariectomized rat model.

Qiao, L., et al. (2008). "NF-kappaB downregulation may be involved the depression of tumor cell proliferation mediated by human mesenchymal stem cells." Acta Pharmacol Sin **29**(3): 333-340.

AIM: It has been reported that stem cells are able to home to tumorigenesis and inhibit the proliferation of tumor cells. The purpose of our study was to demonstrate the molecular mechanism of the inhibitory proliferation of hepatoma cells and breast cancer cells mediated by human mesenchymal stem cells (hMSCs). METHODS: The proliferation of H7402 human hepatoma cells and MCF-7 human breast cancer cells was measured by the 5-bromodeoxyuridine (BrdU) incorporation assay and flow cytometry assay after the treatment with conditioned media from hMSCs culture, such as Z3 cells or BMMS-03 cells. The role of NF-kappaB or the phosphorylation of inhibitor kappaBalpha (p-IkappaBalpha) in the depression of hepatoma or breast cancer cells treated with conditioned media from Z3 cells or BMMS-03 cells was examined by reporter gene assay, quantitative real-time PCR, and Western blot analysis, respectively. RESULTS: The proliferation of H7402 cells and MCF-7 cells was decreased significantly by the BrdU incorporation assay and flow cytometry assay after treatment. The transcriptional activity and mRNA level of NF-kappaB were downregulated in the treated cells by reporter gene assay and quantitative real-time PCR in a dose-dependent manner. At the protein level, NF-kappaB and p-IkappaBalpha decreased in the treated cells by Western blot analysis. CONCLUSION: Conditioned media from hMSCs are able to inhibit the proliferation of tumor cells. NF-kappaB downregulation is one of reasons for the depression of tumor cell proliferation mediated by hMSCs.

Qin, Y., et al. (2017). "Human Embryonic Stem Cell-Derived Mesenchymal Stromal Cells Decrease the Development of Severe Experimental Autoimmune Uveitis in B10.RIII Mice." Ocul Immunol Inflamm: 1-9.

PURPOSE: We investigated the effect of exogenously administered human embryonic stem cell-derived mesenchymal stromal cells (hESC-MSCs) in experimental autoimmune uveitis (EAU) in B10.RIII mice, a murine model of severe uveitis. METHODS: B10.RIII mice were immunized with an uveitogenic peptide, and intraperitoneal injections of 5 million hESC-MSCs per animal were given on the same day. Behavioral light sensitivity assays, histological evaluation, cytokine production, and regulatory T cells were analyzed at the peak of the disease. RESULTS: Histological and behavioral evidence demonstrated that early systemic treatment with hESC-MSCs decreases the development of severe EAU in B10.RIII mice. hESC-MSCs suppress Th17 and upregulate Th1 and Th2 responses as well as IL-2 and GM-CSF in splenocytes from hESC-MSC-treated mice. CONCLUSIONS: MSCs that originate from hESC decrease the development of severe EAU in B10.RIII mice, likely through systemic immune modulation. Further investigation is needed to determine any potential effect on active EAU.

Qu, T., et al. (2013). "Targeted cell reprogramming produces analgesic chromaffin-like cells from human mesenchymal stem cells." Cell Transplant **22**(12): 2257-2266.

Transplantation of allogeneic adrenal chromaffin cells demonstrated the promise of favorable outcomes for pain relief in patients. However, there is a very limited availability of suitable human adrenal gland tissues, genetically well-matched donors in particular, to serve as grafts. Xenogeneic materials, such as porcine and bovine adrenal chromaffin cells, present problems; for instance, immune rejection and possible pathogenic contamination are potential issues. To overcome these challenges, we have tested the novel approach of cell reprogramming to reprogram human bone marrow (BM)-derived mesenchymal stem cells (hMSCs) using cellular extracts of porcine chromaffin cells. We produced a new type of cell, chromaffin-like cells, generated from the reprogrammed hMSCs, which displayed a significant increase in expression of human preproenkephalin (hPPE), a precursor for enkephalin opioid peptides, compared to the inherent expression of hPPE in naive hMSCs. The resultant chromaffin-like cells not only expressed the key molecular markers of adrenal chromaffin cells, such as tyrosine hydroxylase (TH) and methionine enkephalin (Met-enkephalin), but also secreted opioid peptide Met-enkephalin in culture. In addition, intrathecal injection of chromaffin-like cells in rats produced significant analgesic effects without using immunosuppressants. These results suggest that analgesic chromaffin-like cells can be produced from an individual's own tissue-derived stem cells by targeted cell reprogramming and also that these chromaffin-like cells may serve as potential autografts for chronic pain management.

Quang, T., et al. (2014). "Dosage and cell line dependent inhibitory effect of bFGF supplement in human pluripotent stem cell culture on inactivated human mesenchymal stem cells." PLoS One **9**(1): e86031.

Many different culture systems have been developed for expanding human pluripotent stem cells (hESCs and hiPSCs). In general, 4-10 ng/ml of bFGF is supplemented in culture media in feeder-dependent systems regardless of feeder cell types, whereas in feeder-free systems, up to 100 ng/ml of bFGF is required for maintaining long-term culture on various substrates. The amount of bFGF required in native hESCs growth niche is unclear. Here we report using

inactivated adipose-derived human mesenchymal stem cells as feeder cells to examine long-term parallel cultures of two hESCs lines (H1 and H9) and one hiPSCs line (DF19-9-7T) in media supplemented with 0, 0.4 or 4 ng/ml of bFGF for up to 23 passages, as well as parallel cultures of H9 and DF19 in media supplemented with 4, 20 or 100 ng/ml bFGF for up to 13 passages for comparison. Across all cell lines tested, bFGF supplement demonstrated inhibitory effect over growth expansion, single cell colonization and recovery from freezing in a dosage dependent manner. In addition, bFGF exerted differential effects on different cell lines, inducing H1 and DF19 differentiation at 4 ng/ml or higher, while permitting long-term culture of H9 at the same concentrations with no apparent dosage effect. Pluripotency was confirmed for all cell lines cultured in 0, 0.4 or 4 ng/ml bFGF excluding H1-4 ng, as well as H9 cultured in 4, 20 and 100 ng/ml bFGF. However, DF19 demonstrated similar karyotypic abnormality in both 0 and 4 ng/ml bFGF media while H1 and H9 were karyotypically normal in 0 ng/ml bFGF after long-term culture. Our results indicate that exogenous bFGF exerts dosage and cell line dependent effect on human pluripotent stem cells cultured on mesenchymal stem cells, and implies optimal use of bFGF in hESCs/hiPSCs culture should be based on specific cell line and its culture system.

Rajan, T. S., et al. (2017). "Conditioned medium from human gingival mesenchymal stem cells protects motor-neuron-like NSC-34 cells against scratch-injury-induced cell death." Int J Immunopathol Pharmacol **30**(4): 383-394.

Neuronal cell death is a normal process during central nervous system (CNS) development and is also involved in the death of motor neurons in diverse spinal motor neuron degenerative diseases. Here, we investigated the neuroprotective effect of secretory factors released from human gingival mesenchymal stem cells (hGMSCs) in mechanically injured murine motor-neuron-like NSC-34 cells. The cells were exposed to scratch injury and the markers for apoptosis and oxidative stress were examined. Immunocytochemistry results showed that proapoptotic markers cleaved caspase-3 and Bax were elevated while anti-apoptotic protein Bcl-2 was suppressed in scratch-injured NSC-34 cells. Oxidative stress markers SOD-1, inducible nitric oxide synthase (iNOS), Cox-2, and proinflammatory cytokine tumor necrosis factor alpha (TNF-alpha) were activated. Conditioned medium (CM) derived from hGMSCs (hGMSC-CM) significantly blocked the cell death by suppressing SOD-1, iNOS, TNF-alpha, cleaved caspase-3, and Bax. Bcl-2 and anti-inflammatory cytokine anti-interleukin 10 (IL-10) were increased in hGMSC-CM-treated injured cells. Moreover, hGMSC-CM treatment upregulated neurotrophins anti-brain-derived neurotrophic factor (BDNF) and NT3. Western blot data of hGMSC-CM revealed the presence of neurotrophins nerve growth factor (NGF), NT3, anti-inflammatory cytokines IL-10, and transforming growth factor beta (TGF-beta), suggesting their positive role to elicit neuroprotection. Our results propose that hGMSC-CM may serve as a simple and potential autologous therapeutic tool to treat motor neuron injury.

Ramasamy, R., et al. (2008). "The immunosuppressive effects of human bone marrow-derived mesenchymal stem cells target T cell proliferation but not its effector function." Cell Immunol **251**(2): 131-136.

Mesenchymal stem cells (MSC) are non-haematopoietic stem cells that are capable of differentiating into tissues of mesodermal origin. MSC play an important role in supporting the development of fetal and adult haematopoiesis. More recently, MSC have also been found to exhibit inhibitory effect on T cell responses. However, there is little information on the mechanism of this immunosuppression and our study addresses this issue by targeting T cell functions at various level of immune responses. We have generated MSC from human adult bone marrow (BM) and investigated their immunoregulatory function at different phases of T cell responses. MSC showed the ability to inhibit mitogen (CD3/CD28 microbeads)-activated T cell proliferation in a dose-dependent manner. In order to evaluate the specificity of this immunosuppression, the proliferation of CD4(+) and CD8(+) cells were measured. MSC equally inhibit CD4(+) and CD8(+) subpopulations of T cells in response to PHA stimulation. However, the antiproliferative effect of MSC is not due to the inhibition of T cell activation. The expression of early activation markers of T cells, namely CD25 and CD69 were not significantly altered by MSC at 24, 48 and 72h.

Ramos-Mejia, V., et al. (2012). "Maintenance of human embryonic stem cells in mesenchymal stem cell-conditioned media augments hematopoietic specification." Stem Cells Dev **21**(9): 1549-1558.

The realization of human embryonic stem cells (hESC) as a model for human developmental hematopoiesis and in potential cell replacement strategies relies on an improved understanding of the extrinsic and intrinsic factors regulating hematopoietic-specific hESC differentiation. Human mesenchymal stem cells (hMSCs) are multipotent cells of mesodermal origin that form a part of hematopoietic stem cell niches and have an important role in the regulation of hematopoiesis through production of secreted factors and/or cell-to-cell interactions. We

have previously shown that hESCs may be successfully maintained feeder free using hMSC-conditioned media (MSC-CM). Here, we hypothesized that hESCs maintained in MSC-CM may be more prone to differentiation toward hematopoietic lineage than hESCs grown in standard human foreskin fibroblast-conditioned media. We report that specification into hemogenic progenitors and subsequent hematopoietic differentiation and clonogenic progenitor capacity is robustly enhanced in hESC lines maintained in MSC-CM. Interestingly, co-culture of hESCs on hMSCs fully abrogates hematopoietic specification of hESCs, thus suggesting that the improved hematopoietic differentiation is mediated by MSC-secreted factors rather than by MSC-hESC physical interactions. To investigate the molecular mechanism involved in this process, we analyzed global (LINE-1) methylation and genome-wide promoter DNA methylation.

Reitinger, S., et al. (2012). "Electric impedance sensing in cell-substrates for rapid and selective multipotential differentiation capacity monitoring of human mesenchymal stem cells." Biosens Bioelectron **34**(1): 63-69.

Biosensor systems which enable impedance measurements on adherent cell layers under label-free conditions are considered powerful tools for monitoring specific biological characteristics. A radio frequency identification-based sensor platform was adopted to characterize cultivation and differentiation of human bone marrow-derived multipotent stem cells (bmMSC) over periods of up to several days and weeks. Electric cell-substrate impedance sensing was achieved through fabrication of sensitive elements onto glass substrates which comprised two comb-shaped interdigitated gold electrodes covering an area of 1.8 mmx2 mm. The sensing systems were placed into the wells of a 6-well tissue culture plate, stacked onto a reader unit and could thus be handled and operated under sterile conditions. Continuous measurements were carried out with a sinusoidal voltage of 35 mV at a frequency of 10 kHz. After seeding of human bmMSC, this sensor was able to trace significant impedance changes contingent upon cell spreading and adhesion. The re-usable system was further proven suitable for live examination of cell-substrate attachment or continuous cell monitoring up to several weeks. Induction of either osteogenic or adipogenic differentiation could be validated in bmMSC cultures within a few days, in contrast to state-of-the-art protocols, which require several weeks of cultivation time. In the context of medical cell production in a GMP-compliant process, the here presented interdigitated electric microsensor technology allows the documentation of MSC quality in a fast, efficient and reliable fashion.

Ren, H., et al. (2016). "Comparative Analysis of Human Mesenchymal Stem Cells from Umbilical Cord, Dental Pulp, and Menstrual Blood as Sources for Cell Therapy." Stem Cells Int **2016**: 3516574.

Although mesenchymal stem cells (MSCs) based therapy has been considered as a promising tool for tissue repair and regeneration, the optimal cell source remains unknown. Umbilical cord (UC), dental pulp (DP), and menstrual blood (MB) are easily accessible sources, which make them attractive candidates for MSCs. The goal of this study was to compare the biological characteristics, including morphology, proliferation, antiapoptosis, multilineage differentiation capacity, and immunophenotype of UC-, DP-, and MB-MSCs in order to provide a theoretical basis for clinical selection and application of these cells. As a result, all UC-, DP-, and MB-MSCs have self-renewal capacity and multipotentiality. However, the UC-MSCs seemed to have higher cell proliferation ability, while DP-MSCs may have significant advantages for osteogenic differentiation, lower cell apoptosis, and senescence. These differences may be associated with the different expression level of cytokines, including vascular endothelial growth factor, fibroblast growth factor, keratinocyte growth factor, and hepatocyte growth factor in each of the MSCs. Comprehensively, our results suggest DP-MSCs may be a desired source for clinical applications of cell therapy.

Richardson, S. M., et al. (2008). "Human mesenchymal stem cell differentiation to NP-like cells in chitosan-glycerophosphate hydrogels." Biomaterials **29**(1): 85-93.

Intervertebral disc (IVD) degeneration is one of the major causes of low back pain. As current clinical treatments are aimed at restoring biomechanical function and providing symptomatic relief, interest in methods focused on biological repair has increased. Several tissue engineering approaches using different cell types and hydrogels/scaffolds have been proposed. Owing to the unsuitable nature of degenerate cells for tissue engineering attention has focused on the use of mesenchymal stem cells (MSCs). Additionally, while rigid scaffolds have been demonstrated to allow MSC differentiation to the chondrocyte-like cells of the IVD, hydrogels are being increasingly studied as they allow minimally invasive implantation without extensive damage to the IVD. Here, we have studied the temperature-sensitive hydrogel chitosan-glycerophosphate (C/Gp), seeded with human MSCs and cultured for 4 weeks in standard medium. We have analysed the gene and protein expression profile of the MSCs and compared it to that

of both nucleus pulposus (NP) cells and articular chondrocytes cultured in C/Gp.

Riekstina, U., et al. (2009). "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis." Stem Cell Rev **5**(4): 378-386.

Mesenchymal stem cells (MSCs) have been isolated from a variety of human tissues, e.g., bone marrow, adipose tissue, dermis, hair follicles, heart, liver, spleen, dental pulp. Due to their immunomodulatory and regenerative potential MSCs have shown promising results in preclinical and clinical studies for a variety of conditions, such as graft versus host disease (GvHD), Crohn's disease, osteogenesis imperfecta, cartilage damage and myocardial infarction. MSC cultures are composed of heterogeneous cell populations. Complications in defining MSC arise from the fact that different laboratories have employed different tissue sources, extraction, and cultivation methods. Although cell-surface antigens of MSCs have been extensively explored, there is no conclusive evidence that unique stem cells markers are associated with these adult cells. Therefore the aim of this study was to examine expression of embryonic stem cell markers Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4 in adult mesenchymal stem cell populations derived from bone marrow, adipose tissue, dermis and heart. Furthermore, we tested whether human mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions. We found that bone marrow MSCs express embryonic stem cell markers Oct4, Nanog, alkaline phosphatase and SSEA-4, adipose tissue and dermis MSCs express Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4, whereas heart MSCs express Oct4, Nanog, SOX2 and SSEA-4. Our results also indicate that human adult mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions during early passages, as shown by distinct germ layer and embryonic stem cell marker expression patterns. Studies are now needed to determine the functional role of embryonic stem cell markers Oct4, Nanog and SOX2 in adult human MSCs.

Rizvanov, A. A., et al. (2010). "Interaction and self-organization of human mesenchymal stem cells and neuro-blastoma SH-SY5Y cells under co-culture conditions: A novel system for modeling cancer cell micro-environment." Eur J Pharm Biopharm **76**(2): 253-259.

The common drawback of many in vitro cell culture systems is the absence of appropriate micro-environment, which is formed by the combination of factors such as cell-cell contacts, extracellular matrix and paracrine regulation. Micro-environmental factors in a tumor tissue can influence physiological status of the cancer cells and their susceptibility to anticancer therapies. Interaction of cancer cells with their micro-environment and regional stem cells, therefore, is of particular interest. Development of in vitro systems which allow more accurate modeling of complex relations occurring in real tumor environments can increase efficiency of preclinical assays for screening anticancer drugs. The aim of this work was to study interactions between human mesenchymal stem cells (MSCs) and neuro-blastoma cancer SH-SY5Y cells under co-culture conditions on different coated surfaces to determine the effect of co-existence of cancer and stem cells on each cellular population under various stress conditions. We developed an efficient in vitro system for studying individual cancer and stem cell populations during co-culture using differential live fluorescent membrane labeling, and demonstrated self-organization of cancer and stem cells during co-culture on various coated surfaces. Our findings support the evidence that cancer and stem cell interactions play important roles in cellular behavior of cancer cells. These properties can be used in different fields of cancer research, tissue engineering and biotechnology.

Roche, S., et al. (2009). "Comparative proteomic analysis of human mesenchymal and embryonic stem cells: towards the definition of a mesenchymal stem cell proteomic signature." Proteomics **9**(2): 223-232.

Mesenchymal stem cells (MSC) are adult multipotential progenitors which have a high potential in regenerative medicine. They can be isolated from different tissues throughout the body and their homogeneity in terms of phenotype and differentiation capacities is a real concern. To address this issue, we conducted a 2-DE gel analysis of mesenchymal stem cells isolated from bone marrow (BM), adipose tissue, synovial membrane and umbilical vein wall. We confirmed that BM and adipose tissue derived cells were very similar, which argue for their interchangeable use for cell therapy. We also compared human mesenchymal to embryonic stem cells and showed that umbilical vein wall stem cells, a neo-natal cell type, were closer to BM cells than to embryonic stem cells. Based on these proteomic data, we could propose a panel of proteins which were the basis for the definition of a mesenchymal stem cell proteomic signature.

Rodini, C. O., et al. (2018). "Mesenchymal stem cells enhance tumorigenic properties of human glioblastoma through independent cell-cell communication mechanisms." Oncotarget **9**(37): 24766-24777.

Mesenchymal stem cells (MSC) display tumor tropism and have been addressed as vehicles for delivery of anti-cancer agents. As cellular components

of the tumor microenvironment, MSC also influence tumor progression. However, the contribution of MSC in brain cancer is not well understood since either oncogenic or tumor suppressor effects have been reported for these cells. Here, MSC were found capable of stimulating human Glioblastoma (GBM) cell proliferation through a paracrine effect mediated by TGFB1. Moreover, when in direct cell-cell contact with GBM cells, MSC elicited an increased proliferative and invasive tumor cell behavior under 3D conditions, as well as accelerated tumor development in nude mice, independently of paracrine TGFB1. A secretome profiling of MSC-GBM co-cultures identified 126 differentially expressed proteins and 10 proteins exclusively detected under direct cell-cell contact conditions. Most of these proteins are exosome cargos and are involved in cell motility and tissue development.

Sabri, A., et al. (2011). "Crosstalk of EGF-directed MAPK signalling pathways and its potential role on EGF-induced cell proliferation and COX-2 expression in human mesenchymal stem cells." Cell Biochem Funct **29**(1): 64-70.

Epidermal growth factor (EGF) promotes proliferation in human mesenchymal stem cells (hMSCs) during in vitro propagation. In this study, we investigated the effects of PI3K/AKT, ERK1/2, P38 and JNK on EGF signalling in hMSCs. The effects of EGF on MAPKs and PI3K/AKT crosstalk were investigated by immunoblotting; cyclooxygenase-2 (COX-2) expression was studied by real-time RT-PCR; and cell proliferation was evaluated by methylthiazolyl tetrazolium bromide assay. Our results showed that EGF immediately activated all four pathways, induced proliferation and increased COX-2 expression. Interestingly, inhibition of PI3K/AKT-enhanced EGF-stimulated ERK1/2 activity, and inhibition of ERK1/2 and JNK reduced AKT phosphorylation. Furthermore, EGF-induced proliferation as well as EGF-augmented COX2 expression was hindered by ERK1/2 and p38 inhibitors. The results of this study provide evidences to be used in extended proliferation of hMSCs for cell therapy.

Saito, S., et al. (2011). "Use of BAC array CGH for evaluation of chromosomal stability of clinically used human mesenchymal stem cells and of cancer cell lines." Hum Cell **24**(1): 2-8.

Array-based comparative genomic hybridization (aCGH) using bacterial artificial chromosomes (BAC) is a powerful method to analyze DNA copy number aberrations of the entire human genome. In fact, CGH and aCGH have revealed various DNA copy number aberrations in numerous cancer cells and cancer cell lines examined so far. In this report, BAC aCGH was applied to evaluate the stability or instability of cell lines. Established cell lines have greatly contributed to advancements in not only biology but also medical science. However, cell lines have serious problems, such as alteration of biological properties during long-term cultivation. Firstly, we investigated two cancer cell lines, HeLa and Caco-2. HeLa cells, established from a cervical cancer, showed significantly increased DNA copy number alterations with passage time. Caco-2 cells, established from a colon cancer, showed no remarkable differences under various culture conditions. These results indicate that BAC aCGH can be used for the evaluation and validation of genomic stability of cultured cells. Secondly, BAC aCGH was applied to evaluate and validate the genomic stabilities of three patient's mesenchymal stem cells (MSCs), which were already used for their treatments. These three MSCs showed no significant differences in DNA copy number aberrations over their entire chromosomal regions. Therefore, BAC aCGH is highly recommended for use for a quality check of various cells before using them for any kind of biological investigation or clinical application.

Sakaguchi, Y., et al. (2005). "Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source." Arthritis Rheum **52**(8): 2521-2529.

OBJECTIVE: To compare the properties of human mesenchymal stem cells (MSCs) isolated from bone marrow, synovium, periosteum, skeletal muscle, and adipose tissue. METHODS: Human mesenchymal tissues were obtained from 8 donors during knee surgery for ligament injury. After collagenase digestion or gradient-density separation, nucleated cells were plated at an appropriate density for expansion at the maximum rate without colony-to-colony contact. Yield, expandability, differentiation potential, and epitope profile were compared among MSCs from the 5 different tissue sources. RESULTS: Colony number per 10(3) nucleated cells was lower, and cell number per colony was higher, in bone marrow than in other mesenchymal tissues. When the cells were replated at low density every 14 days, bone marrow-, synovium-, and periosteum-derived cells retained their proliferation ability even at passage 10. In chondrogenesis studies in which the cells were pelleted and cultured in vitro, pellets from bone marrow-, synovium-, and periosteum-derived cells were shown to be larger and stained more extensively for cartilage matrix. Synovium-derived cells, in particular, had the greatest ability for chondrogenesis. In adipogenesis experiments, the frequency of oil red O-positive colonies was highest in synovium- and adipose tissue-derived cells. In studies of osteogenesis, the rate of

alizarin red-positive colonies was highest in bone marrow-, synovium-, and periosteum-derived cells. For epitope profiling, 15 surface antigens were measured. Most appeared to have similar epitope profiles irrespective of cell source. CONCLUSION: Our findings indicate that there are significant differences in MSC properties according to tissue source, beyond donor and experimental variation. Superiority of synovium as a potential source of MSCs for clinical applications was demonstrated.

Saleh, F. A., et al. (2011). "Effects of endothelial cells on human mesenchymal stem cell activity in a three-dimensional in vitro model." Eur Cell Mater **22**: 242-257; discussion 257.

An increasing body of data suggest that mesenchymal stem cells (MSCs) reside in a perivascular niche. To more closely mimic this in vivo microenvironment and for better understanding of its complexity, and the factors that regulate the MSC activity, human umbilical vein endothelial cells (HUVECs) were co-cultured with human bone marrow MSCs--using a novel three-dimensional (3D) spheroid co-culture system. Using confocal microscopy of fluorescently labelled cells, we observed HUVECs and MSCs to self-assemble and form organised structures with segregated cell-type partitioning. Under osteogenic conditions, the rate and extent of differentiation in MSC/HUVEC spheroids was significantly elevated compared to 3D co-cultures of MSCs and human dermal fibroblast controls as shown by alkaline phosphatase staining. Conversely, HUVECs inhibited adipogenic differentiation and the proliferation of MSCs in 3D co-cultures indicating that HUVECs suppressed MSC cycling and selectively promoted osteogenic differentiation in 3D. We have also shown that HUVECs enhanced activation of endogenous Wnt signalling and bone morphogenetic protein (BMP) signalling as shown by increased levels of active nuclear beta-catenin and pSmad 1/5/8 immunopositivity respectively. These data suggest strongly that endothelial cells regulate the MSC activity in simulated in vivo conditions, by maintaining quiescence and facilitating niche exit via osteogenic differentiation following appropriate cues. Our findings also underline the importance of 3D heterotypic cell-cell interactions in the regulation of MSC behaviour, suggesting that multicellular cocktails and/or 3D-based delivery strategies may be beneficial for bone repair.

Sardesai, V. S., et al. (2017). "Avoidance of Maternal Cell Contamination and Overgrowth in Isolating Fetal Chorionic Villi Mesenchymal Stem Cells from Human Term Placenta." Stem Cells Transl Med **6**(4): 1070-1084.

Human placenta is rich in mesenchymal stem/stromal cells (MSC), with their origin widely presumed fetal. Cultured placental MSCs are confounded by a high frequency of maternal cell contamination. Our recent systematic review concluded that only a small minority of placental MSC publications report fetal/maternal origin, and failed to discern a specific methodology for isolation of fetal MSC from term villi. We determined isolation conditions to yield fetal and separately maternal MSC during ex vivo expansion from human term placenta. MSCs were isolated via a range of methods in combination; selection from various chorionic regions, different commercial media, mononuclear cell digest and/or explant culture. Fetal and maternal cell identities were quantitated in gender-discordant pregnancies by XY chromosome fluorescence in situ hybridization. We first demonstrated reproducible maternal cell contamination in MSC cultures from all chorionic anatomical locations tested. Cultures in standard media rapidly became composed entirely of maternal cells despite isolation from fetal villi. To isolate pure fetal cells, we validated a novel isolation procedure comprising focal dissection from the cotyledonary core, collagenase/dispase digestion and explant culture in endothelial growth media that selected, and provided a proliferative environment, for fetal MSC. Comparison of MSC populations within the same placenta confirmed fetal to be smaller, more osteogenic and proliferative than maternal MSC. We conclude that in standard media, fetal chorionic villi-derived MSC (CV-MSC) do not grow readily, whereas maternal MSC proliferate to result in maternal overgrowth during culture. Instead, fetal CV-MSCs require isolation under specific conditions, which has implications for clinical trials using placental MSC. Stem Cells Translational Medicine 2017;6:1070-1084.

Sarmadi, V. H., et al. (2008). "The effect of human mesenchymal stem cells on tumour cell proliferation." Med J Malaysia **63 Suppl A**: 63-64.

The therapeutic effect of mesenchymal stem cells (MSC) has been extensively investigated in recent decades, however this therapeutic effect has not been fully characterised. The aim of this study is to elucidate the inhibitory effect of MSC on haematopoietic tumour cells proliferation such as BV173 cell line. To this end, MSC generated from bone marrow, after immunophenotyping, they were co-cultured with tumour cell. The result shows that MSC profoundly inhibit the tumour cell proliferation via arresting the tumour cells at G0 and G1 phase of cell cycle.

Sato, Y., et al. (2013). "Xeno-free and shrinkage-free preparation of scaffold-free cartilage-like disc-shaped

cell sheet using human bone marrow mesenchymal stem cells." J Biosci Bioeng **116**(6): 734-739.

Aiming for the clinical application of cartilage regeneration, the xeno-free cultivation method to obtain a scaffold-free cartilage-like disc-shaped cell sheet using mesenchymal stem cells (MSCs) derived from human bone marrow without the shrinkage of the sheet was investigated. MSCs were inoculated into Cell Culture Insert (0.3 cm(2), pore size; 0.4 mum, pore density; 1.0 x 10(8)/cm(2)) using serum-free chondrogenic differentiation medium containing TGF-beta3, IGF-1 and dexamethasone or other modified media, and cultured at 37 degrees C in 5% CO2 for 3 weeks. Sheet thickness, cartilage specific genes expression, ECM accumulation were determined, and the sections of sheets were stained with alcian blue. A novel mixed medium consisting of a growth medium (10% FCS) with a serum-free chondrogenic differentiation medium could prevent the shrinkage of the sheet and produced a disc-shaped cell sheet. The depth of the sheet was approximately 0.7 mm and the gene expression levels were higher than those in cells in normal human cartilage. The use of human serum instead of FCS did not cause shrinkage and did not decrease the accumulation levels of sGAG and type 2 collagen in the sheet. The cultivation of MSCs grown with completely xeno-free materials using the mixed medium containing human serum in a cell culture insert showed a sheet depth of 1.0 mm and gene expression levels higher than those in normal cartilage. The scaffold-free and xeno-free cartilage-like cell sheet was successfully formed without shrinkage using human bone marrow MSCs and the chondrogenic differentiation medium containing human serum.

Sawada, R., et al. (2006). "Changes in expression of genes related to cell proliferation in human mesenchymal stem cells during in vitro culture in comparison with cancer cells." J Artif Organs **9**(3): 179-184.

We investigated the expression levels of several genes related to cell proliferation in human mesenchymal stem cells (hMSCs) during in vitro culture for use in clinical applications. In this study, we focused on the relationship between hMSC proliferation and transforming growth factor beta (TGFbeta) signaling during in vitro culture. The proliferation rate of hMSCs gradually decreased and marked changes in hMSC morphology were not observed in 3 months of in vitro culture. The mRNA expressions of TGFbeta1, TGFbeta2, and TGFbeta receptor type I (TGFbetaRI) in hMSCs increased with the length of cell culture. There had been no change in the TGFbeta3, TGFbetaRII, and TGFbetaRIII mRNA expressions by the 12th passage from the primary culture (at about 3 months). The mRNA expression of Smad3 increased, but those of c-myc and nucleostemin decreased with the length of hMSC in vitro culture. In addition, the expression profiles of the genes that regulate cellular proliferation in hMSCs were significantly different from those of cancer cells. In conclusion, hMSCs derived from bone marrow seldom underwent spontaneous transformation during 1-2 months of in vitro culture for use in clinical applications. In hMSCs as well as in epithelial cells, growth might be controlled by the TGFbeta family signaling.

Scavo, L. M., et al. (2004). "Insulin-like growth factor-I stimulates both cell growth and lipogenesis during differentiation of human mesenchymal stem cells into adipocytes." J Clin Endocrinol Metab **89**(7): 3543-3553.

Insulin is known to regulate adipocyte differentiation and lipid accumulation, but the specific mechanism by which precursor cells differentiate into adipocytes is not clearly understood. This study evaluated the role of the IGF-I receptor in the process of adipocyte differentiation in bone marrow-derived human mesenchymal stem cells (HMSCs). The results demonstrated that nanomolar concentrations of IGF-I adequately replaced micromolar concentrations of insulin in supporting differentiation and lipid accumulation in HMSCs. The addition of IGF-I specifically increased cell proliferation and lipid accumulation in HMSCs, but a mixture of differentiation factors including dexamethasone, indomethacin, and 3-isobutyl-1-methylxanthine did not. These effects were blocked by the alphaIR-3 antibody, which inhibits IGF-I receptor activity. We also describe the pattern of differentiation with regard to cell growth, lipid accumulation, and morphologic changes and define the changes in these parameters that are influenced by IGF-I. Finally, peroxisome proliferator activating receptor-gamma immunoreactivity was also increased in response to IGF-I, and this effect was blocked in cells treated with the alphaIR-3 antibody. Taken together, these findings suggest that IGF-I plays a critical role in adipocyte differentiation and lipid accumulation.

Scherzad, A., et al. (2015). "Human mesenchymal stem cells enhance cancer cell proliferation via IL-6 secretion and activation of ERK1/2." Int J Oncol **47**(1): 391-397.

Human mesenchymal stem cells (hMSC) are frequently used in tissue engineering. Due to their strong tumor tropism, hMSC seem to be a promising vehicle for anticancer drugs. However, interactions between hMSC and cancer are ambiguous. Particularly the cytokines and growth factors seem to play an important role in cancer progression and metastasis. The present study evaluated the effects of hMSC on

head and neck squamous cell carcinoma (HNSCC) cell lines (FaDu and HLaC78) in vitro. hMSC released several cytokines and growth factors. FaDu and HLaC78 showed a significant enhancement of cell proliferation after cultivation with hMSC-conditioned medium as compared to control. This proliferation improvement was inhibited by the addition of anti-IL-6. The western blot showed an activation of Erk1/2 in FaDu and HLaC78 by hMSC-conditioned medium. HNSCC cell lines expressed EGFR. The current study confirms the importance of cytokines secreted by hMSC in cancer biology. Especially IL-6 seems to play a key role in cancer progression. Thus, the use of hMSC as a carrier for cancer therapy must be discussed critically. Future studies should evaluate the possibility of generating genetically engineered hMSC with, for example, the absence of IL-6 secretion.

Schieker, M., et al. (2007). "Human mesenchymal stem cells at the single-cell level: simultaneous seven-colour immunofluorescence." J Anat **210**(5): 592-599.

Extracellular, intracellular or surface proteins can be used as putative markers to characterize human mesenchymal stem cells (hMSC). However, these markers are also expressed by other cell types and primary cell pools reveal considerable heterogeneity. Therefore, the simultaneous detection of several markers on a single cell appears to be an attractive approach to identify hMSC. Here we demonstrate the specific distinction of human MSC from human osteoblasts via seven-colour fluorescence on the single cell level with simultaneous marker detection of CD44, CD105/endoglin, CD106/VCAM-1, collagen-IV, fibronectin, actin and DAPI nuclear staining. We performed spectral image acquisition using a Sagnac-type interferometer. Subsequent linear unmixing allowed for decomposition of each pixel in its spectral components. Our approach reveals a typical expression profile of the adherent singular cells, allowing the specific distinction between hMSC and osteoblasts on the single cell level.

Schulze, J., et al. (2017). "Effect of hyperbaric oxygen on BDNF-release and neuroprotection: Investigations with human mesenchymal stem cells and genetically modified NIH3T3 fibroblasts as putative cell therapeutics." PLoS One **12**(5): e0178182.

Hyperbaric oxygen therapy (HBOT) is a noninvasive widely applied treatment that increases the oxygen pressure in tissues. In cochlear implant (CI) research, intracochlear application of neurotrophic factors (NTFs) is able to improve survival of spiral ganglion neurons (SGN) after deafness. Cell-based delivery of NTFs such as brain-derived neurotrophic factor (BDNF) may be realized by cell-coating of the surface of the CI electrode. Human mesenchymal stem cells (MSC) secrete a variety of different neurotrophic factors and may be used for the development of a biohybrid electrode in order to release endogenously-derived neuroprotective factors for the protection of residual SGN and for a guided outgrowth of dendrites in the direction of the CI electrode. HBOT could be used to influence cell behaviour after transplantation to the inner ear. The aim of this study was to investigate the effect of HBOT on the proliferation, BDNF-release and secretion of neuroprotective factors. Thus, model cells (an immortalized fibroblast cell line (NIH3T3)-native and genetically modified) and MSCs were repeatedly (3 x - 10 x) exposed to 100% oxygen at different pressures. The effects of HBO on cell proliferation were investigated in relation to normoxic and normobaric conditions (NOR). Moreover, the neuroprotective and neuroregenerative effects of HBO-treated cells were analysed by cultivation of SGN in conditioned medium. Both, the genetically modified NIH3T3/BDNF and native NIH3T3 fibroblasts, showed a highly significant increased proliferation after five days of HBOT in comparison to normoxic controls. By contrast, the number of MSCs was decreased in MSCs treated with 2.0 bar of HBO. Treating SGN cultures with supernatants of fibroblasts and MSCs significantly increased the survival rate of SGN. HBO treatment did not influence (increase / reduce) this effect. Secretome analysis showed that HBO treatment altered the protein expression pattern in MSCs.

Schwab, K. E. and C. E. Gargett (2007). "Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium." Hum Reprod **22**(11): 2903-2911.

BACKGROUND: Human endometrium has immense regenerative capacity, growing ~5 mm in 7 days every month. We have previously identified a small population of colony-forming endometrial stromal cells which we hypothesize are mesenchymal stem cells (MSC). The aim of this study was to determine if the co-expression of two perivascular cell markers, CD146 and platelet-derived growth factor-receptor beta (PDGF-Rbeta), will prospectively isolate endometrial stromal cells which exhibit MSC properties, and determine their location in human endometrium. METHODS: Single cell suspensions of human endometrial stromal cells were fluorescence activated cell sorting (FACS) sorted into CD146(+)PDGF-Rbeta(+) and CD146(-)PDGF-Rbeta(-) populations and analysed for colony-forming ability, in vitro differentiation and expression of typical MSC markers. Full thickness human endometrial sections were co-stained for CD146 and PDGF-Rbeta. RESULTS: FACS stromal CD146(+)PDGF-Rbeta(+) stromal cells (1.5% of sorted population) were enriched

for colony-forming cells compared with CD146(-)PDGF-Rbeta(-) cells (7.7 +/- 1.7 versus 0.7 +/- 0.2% P <0.0001), and also underwent differentiation into adipogenic, osteogenic, myogenic and chondrogenic lineages. They expressed MSC phenotypic surface markers and were located near blood vessels. CONCLUSION: This study shows that human endometrium contains a small population of MSC-like cells that may be responsible for its cyclical growth, and may provide a readily available source of MSC for tissue engineering applications.

Seebach, C., et al. (2010). "Comparison of six bone-graft substitutes regarding to cell seeding efficiency, metabolism and growth behaviour of human mesenchymal stem cells (MSC) in vitro." Injury **41**(7): 731-738.

INTRODUCTION: Various synthetic bone-graft substitutes are used commercially as osteoconductive scaffolds in the treatment of bone defects and fractures. The role of bone-graft substitutes is changing from osteoconductive conduits for growth to an delivery system for biologic fracture treatments. Achieving optimal bone regeneration requires biologics (e.g. MSC) and using the correct scaffold incorporated into a local environment for bone regeneration. The need for an unlimited supply with high quality bone-graft substitutes continue to find alternatives for bone replacement surgery. MATERIALS AND METHODS: This in vitro study investigates cell seeding efficiency, metabolism, gene expression and growth behaviour of MSC sown on six commercially clinical available bone-graft substitutes in order to define their biological properties: synthetic silicate-substituted porous hydroxyapatite (Actifuse ABX), synthetic alpha-TCP (Biobase), synthetic beta-TCP (Vitoss), synthetic beta-TCP (Chronos), processed human cancellous allograft (Tutoplast) and processed bovines hydroxyapatite ceramic (Cerabone). 250,000 MSC derived from human bone marrow (n=4) were seeded onto the scaffolds, respectively. On days 2, 6 and 10 the adherence of MSC (fluorescence microscopy) and cellular activity (MTT assay) were analysed. Osteogenic gene expression (cbfa-1) was analysed by RT-PCR and scanning electron microscopy was performed. RESULTS: The highest number of adhering cells was found on Tutoplast (e.g. day 6: 110.0+/-24.0 cells/microscopic field; p<0.05) followed by Chronos (47.5+/-19.5, p<0.05), Actifuse ABX (19.1+/-4.4), Biobase (15.7+/-9.9), Vitoss (8.8+/-8.7) and Cerabone (8.1+/-2.2). MSC seeded onto Tutoplast showed highest metabolic activity and gene expression of cbfa-1. These data are confirmed by scanning electron microscopy. The cell shapes varied from round-shaped cells to wide spread cells and cell clusters, depending on the bone-graft substitutes. Processed human cancellous allograft is a well-structured and biocompatible scaffold for ingrowing MSC in vitro. Of all other synthetical scaffolds, beta-tricalcium phosphate (Chronos) have shown the best growth behaviour for MSC. DISCUSSION: Our results indicate that various bone-graft substitutes influence cell seeding efficiency, metabolic activity and growth behaviour of MSC in different manners. We detected a high variety of cellular integration of MSC in vitro, which may be important for bony integration in the clinical setting.

Seiler, C., et al. (2014). "Time-lapse microscopy and classification of 2D human mesenchymal stem cells based on cell shape picks up myogenic from osteogenic and adipogenic differentiation." J Tissue Eng Regen Med **8**(9): 737-746.

Current methods to characterize mesenchymal stem cells (MSCs) are limited to CD marker expression, plastic adherence and their ability to differentiate into adipogenic, osteogenic and chondrogenic precursors. It seems evident that stem cells undergoing differentiation should differ in many aspects, such as morphology and possibly also behaviour; however, such a correlation has not yet been exploited for fate prediction of MSCs. Primary human MSCs from bone marrow were expanded and pelleted to form high-density cultures and were then randomly divided into four groups to differentiate into adipogenic, osteogenic chondrogenic and myogenic progenitor cells. The cells were expanded as heterogeneous and tracked with time-lapse microscopy to record cell shape, using phase-contrast microscopy. The cells were segmented using a custom-made image-processing pipeline. Seven morphological features were extracted for each of the segmented cells. Statistical analysis was performed on the seven-dimensional feature vectors, using a tree-like classification method. Differentiation of cells was monitored with key marker genes and histology. Cells in differentiation media were expressing the key genes for each of the three pathways after 21 days, i.e. adipogenic, osteogenic and chondrogenic, which was also confirmed by histological staining. Time-lapse microscopy data were obtained and contained new evidence that two cell shape features, eccentricity and filopodia (= 'fingers') are highly informative to classify myogenic differentiation from all others. However, no robust classifiers could be identified for the other cell differentiation paths. The results suggest that non-invasive automated time-lapse microscopy could potentially be used to predict the stem cell fate of hMSCs for clinical application, based on morphology for earlier time-points. The classification is challenged by cell density, proliferation and possible unknown donor-specific factors, which affect the performance of morphology-based approaches.

Seo, S. K., et al. (2013). "Overexpression of human arginine decarboxylase rescues human mesenchymal stem cells against H(2)O(2) toxicity through cell survival protein activation." J Korean Med Sci **28**(3): 366-373.

In this study, we explored the potentiality of human arginine decarboxylase (ADC) to enhance the survival of mesenchymal stem cells (MSCs) against unfavorable milieu of host tissues as the low survival of MSCs is the issue in cell transplantation therapy. To address this, human MSCs overexpressing human ADC were treated with H2O2 and the resultant intracellular events were examined. First, we examined whether human ADC is overexpressed in human MSCs. Then, we investigated cell survival or death related events. We found that the overexpression of human ADC increases formazan production and reduces caspase 3 activation and the numbers of FITC, hoechst, or propidium iodide positive cells in human MSCs exposed to H2O2. To elucidate the factors underlying these phenomena, AKT, CREB, and BDNF were examined. We found that the overexpression of human ADC phosphorylates AKT and CREB and increases BDNF level in human MSCs exposed to H2O2. The changes of these proteins are possibly relevant to the elevation of agmatine. Collectively, our data demonstrate that the overexpression of human ADC stimulates pro-survival factors to protect human MSCs against H2O2 toxicity. In conclusion, the present findings support that ADC can enhance the survival of MSCs against hostile environment of host tissues.

Sequiera, G. L., et al. (2017). "Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells as an Individual-Specific and Renewable Source of Adult Stem Cells." Methods Mol Biol **1553**: 183-190.

This chapter deals with the employment of human-induced pluripotent stem cells (hiPSCs) as a candidate to differentiate into mesenchymal stem cells (MSCs). This would enable to help establish a regular source of human MSCs with the aim of avoiding the problems associated with procuring the MSCs either from different healthy individuals or patients, limited extraction potentials, batch-to-batch variations or from diverse sources such as bone marrow or adipose tissue. The procedures described herein allow for a guided and ensured approach for the regular maintenance of hiPSCs and their subsequent differentiation into MSCs using the prescribed medium. Subsequently, an easy protocol for the successive isolation and purification of the hiPSC-differentiated MSCs is outlined, which is carried out through passaging and can be further sorted through flow cytometry. Further, the maintenance and expansion of the resultant hiPSC-differentiated MSCs using appropriate characterization techniques, i.e., Reverse-transcription PCR and immunostaining is also elaborated. The course of action has been deliberated keeping in mind the awareness and the requisites available to even beginner researchers who mostly have access to regular consumables and medium components found in the general laboratory.

Shahdadfar, A., et al. (2005). "In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability." Stem Cells **23**(9): 1357-1366.

Human bone marrow mesenchymal stem cells (hMSCs) represent an appealing source of adult stem cells for cell therapy and tissue engineering, as they are easily obtained and expanded while maintaining their multilineage differentiation potential. All current protocols for in vitro culture of hMSCs include fetal bovine serum (FBS) as nutritional supplement. FBS is an undesirable additive to cells that are expanded for therapeutic purposes in humans because the use of FBS carries the risk of transmitting viral and prion diseases and proteins that may initiate xenogeneic immune responses. In the present study, we have therefore investigated if autologous serum (AS) or allogeneic human serum (alloHS) could replace FBS for the expansion of hMSCs in vitro. We discovered that the choice of serum affected hMSCs at several different levels. First, hMSCs in AS proliferated markedly faster than hMSCs in FBS, whereas use of alloHS resulted in hMSC growth arrest and death. Second, hMSCs in FBS differentiated more rapidly toward mesenchymal lineages compared with hMSCs in AS. Interestingly, genome-wide microarray analysis identified several transcripts involved in cell cycle and differentiation that were differentially regulated between hMSCs in FBS and AS. Finally, several transcripts, including some involved in cell cycle inhibition, were upregulated in hMSCs in FBS at a late passage, whereas the hMSC transcriptome in AS was remarkably stable. Thus, hMSCs may be expanded rapidly and with stable gene expression in AS in the absence of growth factors, whereas FBS induces a more differentiated and less stable transcriptional profile.

Shan, L. (2004). Multimodal, rhodamine B isothiocyanate-incorporated, silica-coated magnetic nanoparticle-labeled human cord blood-derived mesenchymal stem cells for cell tracking. Molecular Imaging and Contrast Agent Database (MICAD). Bethesda (MD).

Personalized diagnosis and treatment with allogenic or autologous cells are becoming a reality in the field of medicine (1, 2). Cytotoxic or engineered T

cells are under clinical trial for the treatment of hematopoietic or other malignant diseases (1). Contrast agent-tagged macrophages are used as cellular probes to image the early inflammatory processes in macrophage-rich conditions such as inflammation, atherosclerosis, and acute cardiac graft rejection (2). The roles of stem cells are under intensive investigation in the therapeutic and regenerative medicine such as regenerating cardiomyocytes, neurons, bone, and cartilage (3). Genetically modified cells are used to treat genetic disorders (4). With the promising results from these studies, a critical issue is how to monitor the temporal and spatial migration and the homing of these cells, as well as the engraftment efficiency and functional capability of the transplanted cells in vivo (5, 6). Histopathological techniques have only been used to obtain information on the fate of implanted cells at the time of animal euthanization or via biopsy or surgery. To track the real-time changes of cell location, viability, and functional status, cell imaging techniques have been introduced during the last few years. Cells of interest are labeled with reporter genes, fluorescent dyes, or other contrast agents that transform the tagged cells into cellular probes or imaging agents (5-7). The ability to monitor superparamagnetic iron oxide particles (SPIO) by magnetic resonance imaging (MRI) has been utilized in animal models as well as in a few clinical settings to investigate the fate of labeled cells (5-9). The advantages of using MRI for cell tracking include the high spatial resolution with high anatomic background contrast, the lack of exposure to ionizing radiation, and the ability to follow the cells for months. SPIO particles provide a strong change in signal per unit of metal, in particular on T2- and T2*-weight images. In addition, cell labeling with SPIO nanoparticles is generally nontoxic and does not affect the cell proliferation and differentiation capacity, although a few studies have reported that the stem cells labeled with SPIO lose part of their differentiation capacity in a SPIO concentration-dependent manner (8, 9). An important limitation of MRI is the fact that MRI signals cannot indicate whether cells are dead or alive. It is also unknown whether the MRI signal comes from targeted or labeled cells or from macrophages. Basically, SPIO particles are used to label the target cells by systemic application or by injecting into the tissue area of interest to monitor target cell migration after phagocytosis. SPIO are more frequently used to label the cells in vitro by incorporating into the cells directly. Furthermore, SPIO are usually encapsulated by organic polymers to increase their stability and bio-compatibility, and allow the chemical modification of their surfaces. The fact is that the uptake of different particles varies largely between different cell types (5, 6). Mesenchymal stem cells (MSCs) represent a heterogeneous subset of pluripotent stromal cells that can be isolated from different adult tissues including adipose tissue, liver, muscle, amniotic fluid, placenta, umbilical cord blood, and dental pulp, although the bone marrow remains the principal source for most preclinical and clinical studies (3, 10, 11). Although MSCs account for only 0.01-0.001% of the total nucleated cells within isolated bone marrow aspirates, they can easily be isolated and expanded in vitro through as many as 40 population doublings in ~8-10 weeks of culture (12). These cells exhibit the potential to differentiate into cells of diverse lineages, such as adipocytes, chondrocytes, osteocytes, myoblasts, cardiomyocytes, neurons, and astrocytes. In addition, MSCs possess remarkable immunosuppressive properties, and they have been shown to be effective against tumor cell growth (13, 14). Yoon et al. generated multimodal, rhodamine B isothiocyanate (RITC)-labeled, silica-coated magnetic nanoparticles (MNPs@SiO2(RITC)) and Park et al. labeled the MSCs with the nanoparticles and successfully tracked the labeled cells transplanted into the subcutaneous tissue and liver of the mice (7, 15). Their results indicate that MNPs@SiO2(RITC) are biocompatible and useful for human MSC labeling and cell tracking with multimodality imaging.

Shi, G., et al. (2008). "Phenotypic characteristics of hybrid cells produced by cell fusion of porcine adrenal chromaffin cells with human mesenchymal stem cells: a preliminary study." Neurol Res **30**(3): 217-222.

BACKGROUND AND PURPOSE: Transplantation of adrenal chromaffin cells (CCs) that release endogenous opioid peptides and catecholamines produces significant antinociceptive effects in patients with terminal cancer pain. In clinical practice, however, obtaining a sufficient number of chromaffin cells may not be possible because of the limited availability of human adrenal tissue. Recent works have shown that fusion of bone marrow-derived cells with differentiated cells can occur spontaneously in vivo and acquire the phenotype of the recipient cells. In this study, we investigated the possibility of producing chromaffin-like cells by fusing human bone marrow-derived mesenchymal stem cells (MeSCs) with post-mitotic porcine CCs in vitro with the application of polyethylene glycol (PEG). METHODS AND RESULTS: Before cell-to-cell fusion was initiated, MeSCs and CCs were labeled by fluorescence dyes DiO (green) and Dil (red), respectively. The hybrid cells generated by PEG-mediated fusion expressed a DiO and Dil double-staining with estimated fusion efficiency at approximately 40% of the total cell population. Further immunocytochemical examination for tyrosine hydroxylase and methionine enkephalin (markers for CCs) demonstrated positive immuno-reactivity in these hybrid cells 2 weeks post-fusion.

More interestingly, some of the hybrid cells showed bromodeoxyuridine (BrdU)-positive immunostaining in the nuclei. DISCUSSION: Our results show that these hybrid cells fused by CCs and MeSCs express some characteristics of the CC phenotype. A subpopulation of these hybrid cells are dividing cells with positive BrdU immunostaining, suggesting that a novel cellular production could be developed by a "reprogramming" mechanism through the application of targeted cell fusion strategies.

Sohn, H. S., et al. (2013). "Duration of in vitro storage affects the key stem cell features of human bone marrow-derived mesenchymal stromal cells for clinical transplantation." Cytotherapy **15**(4): 460-466.

BACKGROUND AIMS: Mesenchymal stromal cells (MSCs) have the ability to self-renew and differentiate into various cell types. Their plasticity and easy availability make them promising candidates for regenerative medicine. However, for successful clinical application, MSCs need to be expanded under a Good Manufacturing Practices-compliant system to obtain a large quantity of these cells. Although the viability and potency of these in vitro-expanded MSCs need to be maintained during preparation and transportation before transplantation, these characteristics have not thoroughly been examined. Our goal in this study was to standardize MSC preparation and storage before their clinical application to ensure reproducible quality and potency for their clinically intended purpose. METHODS: We examined the viability, self-renewal capacity and differentiation capability of MSCs on short-term in vitro storage in saline or dextrose solution at 4 degrees C and room temperature. RESULTS: MSCs harvested and suspended in saline for 1-2 h showed >90% viability regardless of storage temperature. However, when cells were stored for >2 h in saline, their viability decreased gradually over time. The viability of cells in dextrose deteriorated rapidly. MSCs lost colony-forming unit and differentiation capacities rapidly as storage time increased. Collectively, we found that a storage period >2 h resulted in a significant decrease in cell viability, cell proliferation capacity and differentiation potency. CONCLUSIONS: Storage of culture-harvested MSCs for >2 h is likely to result in suboptimal MSC-mediated tissue regeneration because of decreased cell viability and differentiation capacity.

Somoza, R., et al. (2010). "Intranigral transplantation of epigenetically induced BDNF-secreting human mesenchymal stem cells: implications for cell-based therapies in Parkinson's disease." Biol Blood Marrow Transplant **16**(11): 1530-1540.

It is thought that the ability of human mesenchymal stem cells (hMSC) to deliver neurotrophic factors might be potentially useful for the treatment of neurodegenerative disorders. The aim of the present study was to characterize signals and/or molecules that regulate brain-derived neurotrophic factor (BDNF) protein expression/delivery in hMSC cultures and evaluate the effect of epigenetically generated BDNF-secreting hMSC on the intact and lesioned substantia nigra (SN). We tested 4 different culture media and found that the presence of fetal bovine serum (FBS) decreased the expression of BDNF, whereas exogenous addition of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to serum-free medium was required to induce BDNF release (125 +/- 12 pg/day/10(6) cells). These cells were called hM(N)SC. Although the induction medium inhibited the expression of alpha smooth muscle actin (ASMA), an hMSC marker, and increased the nestin-positive subpopulation of hMSC cultures, the ability to express BDNF was restricted to the nestin-negative subpopulation. One week after transplantation into the SN, the human cells integrated into the surrounding tissue, and some showed a dopaminergic phenotype. We also observed the activation of Trk receptors for neurotrophic factors around the implant site, including the BDNF receptor TrkB. When we transplanted these cells into the unilateral lesioned SN induced by striatal injection of 6-hydroxydopamine (6-OHDA), a significant hypertrophy of nigral tyrosine hydroxylase (TH)(+) cells, an increase of striatal TH-staining and stabilization of amphetamine-induced motor symptoms were observed. Therefore, hMSC cultures exposed to the described induction medium might be highly useful as a vehicle for neurotrophic delivery to the brain and specifically are strong candidates for future therapeutic application in Parkinson's disease.

Son, M., et al. (2017). "Protection against RAGE-mediated neuronal cell death by sRAGE-secreting human mesenchymal stem cells in 5xFAD transgenic mouse model." Brain Behav Immun **66**: 347-358.

Alzheimer's disease (AD), which is the most commonly encountered neurodegenerative disease, causes synaptic dysfunction and neuronal loss due to various pathological processes that include tau abnormality and amyloid beta (Abeta) accumulation. Abeta stimulates the secretion and the synthesis of Receptor for Advanced Glycation End products (RAGE) ligand by activating microglial cells, and has been reported to cause neuronal cell death in Abeta1-42 treated rats and in mice with neurotoxin-induced Parkinson's disease. The soluble form of RAGE (sRAGE) is known to reduce inflammation, and to decrease microglial cell activation and Abeta deposition, and thus, it protects from neuronal cell death in AD. However, sRAGE protein has too a short half-life for therapeutic purposes. We developed

sRAGE-secreting umbilical cord derived mesenchymal stem cells (sRAGE-MSCs) to enhance the inhibitory effects of sRAGE on Abeta deposition and to reduce the secretion and synthesis of RAGE ligands in 5xFAD mice. In addition, these cells improved the viability of injected MSCs, and enhanced the protective effects of sRAGE by inhibiting the binding of RAGE and RAGE ligands in 5xFAD mice. These findings suggest sRAGE protein from sRAGE-MSCs has better protection against neuronal cell death than sRAGE protein or single MSC treatment by inhibiting the RAGE cell death cascade and RAGE-induce inflammation.

Song, C., et al. (2015). "Use of Ferritin Expression, Regulated by Neural Cell-Specific Promoters in Human Adipose Tissue-Derived Mesenchymal Stem Cells, to Monitor Differentiation with Magnetic Resonance Imaging In Vitro." PLoS One **10**(7): e0132480.

The purpose of this study was to establish a method for monitoring the neural differentiation of stem cells using ferritin transgene expression, under the control of a neural-differentiation-inducible promoter, and magnetic resonance imaging (MRI). Human adipose tissue-derived mesenchymal stem cells (hADMSCs) were transduced with a lentivirus containing the human ferritin heavy chain 1 (FTH1) gene coupled to one of three neural cell-specific promoters: human synapsin 1 promoter (SYN1p, for neurons), human glial fibrillary acidic protein promoter (GFAPp, for astrocytes), and human myelin basic protein promoter (MBPp, for oligodendrocytes). Three groups of neural-differentiation-inducible ferritin-expressing (NDIFE) hADMSCs were established: SYN1p-FTH1, GFAPp-FTH1, and MBPp-FTH1. The proliferation rate of the NDIFE hADMSCs was evaluated using a Cell Counting Kit-8 assay. Ferritin expression was assessed with western blotting and immunofluorescent staining before and after the induction of differentiation in NDIFE hADMSCs. The intracellular iron content was measured with Prussian blue iron staining and inductively coupled plasma mass spectrometry. R2 relaxation rates were measured with MRI in vitro. The proliferation rates of control and NDIFE hADMSCs did not differ significantly ($P > 0.05$). SYN1p-FTH1, GFAPp-FTH1, and MBPp-FTH1 hADMSCs expressed specific markers of neurons, astrocytes, and oligodendrocytes, respectively, after neural differentiation. Neural differentiation increased ferritin expression twofold, the intracellular iron content threefold, and the R2 relaxation rate two- to threefold in NDIFE hADMSCs, resulting in notable hypointensity in T2-weighted images ($P < 0.05$). These results were cross-validated. Thus, a link between neural differentiation and MRI signals (R2 relaxation rate) was established in hADMSCs. The use of MRI and neural-differentiation-inducible ferritin expression is a viable method for monitoring the neural differentiation of hADMSCs.

Spitzhorn, L. S., et al. (2018). "Transplanted human pluripotent stem cell-derived mesenchymal stem cells support liver regeneration in Gunn rats." Stem Cells Dev.

Gunn rats bear a mutation within the uridine diphosphate glucuronosyltransferase-1A1 (Ugt1a1) gene resulting in high serum bilirubin levels as seen in Crigler-Najjar syndrome. In the present study, the Gunn rat was used as an animal model for heritable liver dysfunction. Human mesenchymal stem cells (iMSC) derived from embryonic stem cells (H1) and induced pluripotent stem cells were transplanted into Gunn rats after partial hepatectomy. The iMSCs engrafted and survived in the liver for up to 2 months. The transplanted iMSCs differentiated into functional hepatocytes as evidenced by partially suppressed hyperbilirubinemia and expression of multiple human-specific hepatocyte markers such as Albumin, hepatocyte nuclear factor 4alpha, UGT1A1, Cytokeratin 18, bile salt export pump, multidrug resistance protein 2, Na/taurocholate-cotransporting polypeptide and alpha-Fetoprotein. These findings imply that transplanted human iMSCs can contribute to liver regeneration in vivo and thus represent a promising tool for the treatment of inherited liver diseases.

Sriram, G., et al. (2016). "Innate Immune Response of Human Embryonic Stem Cell-Derived Fibroblasts and Mesenchymal Stem Cells to Periodontopathogens." Stem Cells Int **2016**: 8905365.

Periodontitis involves complex interplay of bacteria and host immune response resulting in destruction of supporting tissues of the tooth. Toll-like receptors (TLRs) play a role in recognizing microbial pathogens and eliciting an innate immune response. Recently, the potential application of multipotent stem cells and pluripotent stem cells including human embryonic stem cells (hESCs) in periodontal regenerative therapy has been proposed. However, little is known about the impact of periodontopathogens on hESC-derived progenies. This study investigates the effects of heat-killed periodontopathogens, namely, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, on TLR and cytokine expression profile of hESC-derived progenies, namely, fibroblasts (hESC-Fib) and mesenchymal stem cells (hESC-MSCs). Additionally, the serotype-dependent effect of A. actinomycetemcomitans on hESC-derived progenies was explored. Both hESC-Fib and hESC-MSCs constitutively expressed TLR-2 and TLR-4.

hESC-Fib upon exposure to periodontopathogens displayed upregulation of TLRs and release of cytokines (IL-1beta, IL-6, and IL-8).

Stanco, D., et al. (2015). "Multidifferentiation potential of human mesenchymal stem cells from adipose tissue and hamstring tendons for musculoskeletal cell-based therapy." Regen Med **10**(6): 729-743.

AIM: Adipose-derived stem cells (ASCs) have been deeply characterized for their usefulness in musculoskeletal tissue regeneration; recently, other mesenchymal stem cell (MSC) sources have also been proposed. This study compares for the first time human tendon stem/progenitor cells isolated from hamstring tendons with human ASCs. MATERIALS & METHODS: Human TSPCs and ASCs were isolated from hamstring tendon portions and adipose tissue of healthy donors undergoing ACL reconstruction or liposuction, respectively (n = 7). Clonogenic ability, immunophenotype and multi-differentiation potential were assessed and compared. RESULTS: Both populations showed similar proliferation and clonogenic ability and expressed embryonic stem cell genes and MSC surface markers. Tendon stem/progenitor cells showed lower adipogenic and osteogenic ability, but after the chondrogenic differentiation, they produced more abundant glycosaminoglycans and expressed higher levels of aggrecan with regards to ASCs. The tenogenic induction with BMP-12 upregulated SCX and DCN gene expression in both populations. CONCLUSION: Our results demonstrate that waste hamstring tendon fragments could represent a convenient MSC source for musculoskeletal regenerative medicine.

Steinert, A. F., et al. (2011). "Mesenchymal stem cell characteristics of human anterior cruciate ligament outgrowth cells." Tissue Eng Part A **17**(9-10): 1375-1388.

When ruptured, the anterior cruciate ligament (ACL) of the human knee has limited regenerative potential. However, the goal of this report was to show that the cells that migrate out of the human ACL constitute a rich population of progenitor cells and we hypothesize that they display mesenchymal stem cell (MSC) characteristics when compared with adherent cells derived from bone marrow or collagenase digests from ACL. We show that ACL outgrowth cells are adherent, fibroblastic cells with a surface immunophenotype strongly positive for cluster of differentiation (CD)29, CD44, CD49c, CD73, CD90, CD97, CD105, CD146, and CD166, weakly positive for CD106 and CD14, but negative for CD11c, CD31, CD34, CD40, CD45, CD53, CD74, CD133, CD144, and CD163. Staining for STRO-1 was seen by immunohistochemistry but not flow cytometry. Under suitable culture conditions, the ACL outgrowth-derived MSCs differentiated into chondrocytes, osteoblasts, and adipocytes and showed capacity to self-renew in an in vitro assay of ligamentogenesis. MSCs derived from collagenase digests of ACL tissue and human bone marrow were analyzed in parallel and displayed similar, but not identical, properties. In situ staining of the ACL suggests that the MSCs reside both aligned with the collagenous matrix of the ligament and adjacent to small blood vessels. We conclude that the cells that emigrate from damaged ACLs are MSCs and that they have the potential to provide the basis for a superior, biological repair of this ligament.

Stoddart, M. J. (2011). "WST-8 analysis of cell viability during osteogenesis of human mesenchymal stem cells." Methods Mol Biol **740**: 21-25.

WST-8 is one of the newer generation formazan-based dyes, which release the converted product into the medium in a soluble form. This allows for a non-destructive determination of viability enabling the cells to be subject to further investigations. This is a major advantage in cases where cell phenotype is being investigated and data such as matrix synthesis is correlated to cell number. This chapter describes the use of WST-8 to normalise alkaline phosphatase activity.

Stolzing, A., et al. (2008). "Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies." Mech Ageing Dev **129**(3): 163-173.

Human mesenchymal stem cells (hMSC) represent a promising cell-based therapy for a number of degenerative conditions. Understanding the effect of aging on hMSCs is crucial for autologous therapy development in older subject whom degenerative diseases typically afflict. Previous investigations into the effects of aging on hMSC have proved contradictory due to the relative narrow age ranges of subjects assessed and the exclusive reliance of in vitro assays. This study seeks to address this controversy by using a wider range of donor ages and by measuring indices of cellular aging as well as hMSC numbers ex vivo and proliferation rates. CFU-f analysis and flow cytometry analysis using a CD45(low)/D7fib(+ve)/LNGF(+ve) gating strategy were employed. In addition a variety of markers of cellular aging, oxidative damage and senescence measured. A reduction in CFU-f and CD45(low)/D7fib(+ve)/LNGF(+ve) cell numbers were noted in adulthood relative to childhood. Indices of aging including oxidative damage, ROS levels and p21 and p53 all increased suggesting a loss of MSC fitness with age. These data suggest that hMSC numbers obtained by marrow aspiration decline with age.

Furthermore, there is an age-related decline in overall BM MSC "fitness" which might lead to problems when using autologous aged MSC for cell-based therapies.

Stopp, S., et al. (2013). "Expression of the melanoma cell adhesion molecule in human mesenchymal stromal cells regulates proliferation, differentiation, and maintenance of hematopoietic stem and progenitor cells." Haematologica **98**(4): 505-513.

The melanoma cell adhesion molecule defines mesenchymal stromal cells in the human bone marrow that regenerate bone and establish a hematopoietic microenvironment in vivo. The role of the melanoma cell adhesion molecule in primary human mesenchymal stromal cells and the maintenance of hematopoietic stem and progenitor cells during ex vivo culture has not yet been demonstrated. We applied RNA interference or ectopic overexpression of the melanoma cell adhesion molecule in human mesenchymal stromal cells to evaluate the effect of the melanoma cell adhesion molecule on their proliferation and differentiation as well as its influence on co-cultivated hematopoietic stem and progenitor cells. Knockdown and overexpression of the melanoma cell adhesion molecule affected several characteristics of human mesenchymal stromal cells related to osteogenic differentiation, proliferation, and migration. Furthermore, knockdown of the melanoma cell adhesion molecule in human mesenchymal stromal cells stimulated the proliferation of hematopoietic stem and progenitor cells, and strongly reduced the formation of long-term culture-initiating cells. In contrast, melanoma cell adhesion molecule-overexpressing human mesenchymal stromal cells provided a supportive microenvironment for hematopoietic stem and progenitor cells. Expression of the melanoma cell adhesion molecule increased the adhesion of hematopoietic stem and progenitor cells to human mesenchymal stromal cells and their migration beneath the monolayer of human mesenchymal stromal cells.

Strojny, C., et al. (2015). "Interferon Gamma-treated Dental Pulp Stem Cells Promote Human Mesenchymal Stem Cell Migration In Vitro." J Endod **41**(8): 1259-1264.

INTRODUCTION: Chronic inflammation disrupts dental pulp regeneration by disintegrating the recruitment process of progenitors for repair. Bone marrow-derived mesenchymal stem cells (BM-MSCs) share the common features with dental pulp stem cells (DPSCs). The aim of the study was to investigate the migration of BM-MSCs toward DPSCs in response to inflammatory chemoattractants. Additionally, our studies also delineated the signaling mechanisms from BM-MSCs in mediating the proliferation and differentiation of DPSCs in vitro. METHODS: Human DPSCs and BM-MSCs between passages 2 and 4 were used and were grown in odontogenic differentiation medium. Mineralization was determined by alizarin red staining analysis. Migration was assessed using crystal violet staining in cells grown in Boyden chamber Transwell inserts (Corning Inc Foundation, Tewksbury, MA). The mineralization potential of DPSCs was evaluated using alkaline phosphatase activity assay. Real-time polymerase chain reaction analysis was performed to assess the gene expression profile of chemokine (C-X-C motif) ligand (Cxcl) 3, 5, 6, 10, 11, 12, 14, and 16; stromal cell-derived factor (SDF) alpha; vascular endothelial growth factor; and fibroblast growth factor. RESULTS: Interferon gamma (FN-gamma) treatment significantly abrogated the differentiation potential of DPSCs as shown by using alizarin red and alkaline phosphatase activity analysis. An increase in the migration of BM-MSCs was documented when cocultured with IFN-gamma-treated DPSCs.

Suzuki, S., et al. (2010). "Effects of extracellular matrix on differentiation of human bone marrow-derived mesenchymal stem cells into smooth muscle cell lineage: utility for cardiovascular tissue engineering." Cells Tissues Organs **191**(4): 269-280.

BACKGROUND: Bone marrow-derived mesenchymal stem cells (MSCs) can differentiate into various types of cell, and the extracellular matrix (ECM) is acknowledged to be important for the regulation of cell functions. In this study, we demonstrated the effects of ECMs on the differentiation of human bone marrow-derived MSCs into a smooth muscle cell (SMC) lineage. METHODS: Human MSCs (hMSCs) were cultured on dishes coated with 3 types of ECM including laminin (LM), collagen type IV (Col-IV) and fibronectin for 7 days, and simultaneously cultured on a noncoated dish as a control. Cell numbers of these cultured hMSCs were counted, and their expression of SMC-specific genes and proteins was evaluated. hMSCs were then seeded on LM-coated biodegradable sheets and implanted into rat subcutaneous space. After 2 weeks of implantation, these tissues were evaluated. RESULTS: The number of hMSCs was significantly increased by culture on Col-IV-coated dishes. The expression of SMC-specific genes and proteins (alpha-smooth muscle actin, ASMA; h1-calponin, CALP) in hMSC was significantly upregulated from culture on LM-coated dishes. LM-coated sheets showed a significantly increased expression of ASMA and CALP protein in vivo. Moreover, a fully differentiated marker (SM2) was expressed in the in vivo implanted hMSCs in the course of 2 weeks on the LM-coated sheet. CONCLUSION: These results suggest that the signal

transduction of the cell-matrix interaction for the differentiation of hMSCs into SMCs was activated when cultured with LM. LM-coated materials may thus be useful for cardiovascular tissue engineering.

Sze, S. K., et al. (2007). "Elucidating the secretion proteome of human embryonic stem cell-derived mesenchymal stem cells." Mol Cell Proteomics **6**(10): 1680-1689.

Transplantation of mesenchymal stem cells (MSCs) has been used to treat a wide range of diseases, and the mechanism of action is postulated to be mediated by either differentiation into functional reparative cells that replace injured tissues or secretion of paracrine factors that promote tissue repair. To complement earlier studies that identified some of the paracrine factors, we profiled the paracrine proteome to better assess the relevance of MSC paracrine factors to the wide spectrum of MSC-mediated therapeutic effects. To evaluate the therapeutic potential of the MSC paracrine proteome, a chemically defined serum-free culture medium was conditioned by MSCs derived from human embryonic stem cells using a clinically compliant protocol. The conditioned medium was analyzed by multidimensional protein identification technology and cytokine antibody array analysis and revealed the presence of 201 unique gene products. 86-88% of these gene products had detectable transcript levels by microarray or quantitative RT-PCR assays. Computational analysis predicted that these gene products will significantly drive three major groups of biological processes: metabolism, defense response, and tissue differentiation including vascularization, hematopoiesis, and skeletal development.

Takagi, M., et al. (2007). "High inoculation cell density could accelerate the differentiation of human bone marrow mesenchymal stem cells to chondrocyte cells." J Biosci Bioeng **103**(1): 98-100.

The effects of the density of human mesenchymal stem cells (MSCs) on their differentiation to chondrocytes in a differentiation medium supplemented with dexamethasone, TGFbeta3, and IGF-1 were investigated for the regenerative therapy of cartilage. The increase in the initial density of MSCs from 0.05 x 10(4) to 0.9 x 10(4) cells/cm(2) accelerated the increase in the expression level of aggrecan mRNA during the differentiation culture for 7 d. The conditioned medium harvested at 7 d from the differentiation culture with an initial MSC density of 0.3 x 10(4) cells/cm(2) accelerated the initial increase in the expression level for 3 d in the differentiation culture with an initial MSC density of 0.3 x 10(4) cells/cm(2), whereas the conditioned medium harvested at 7 d in the differentiation culture with an initial MSC density of 0.05 x 10(4) cells/cm(2) did not. The differentiation culture after 14 d with an initial MSC concentration of 0.3 x 10(4) cells/cm(2) showed an expression level 1.7-fold that in the case of the culture with an initial MSC concentration of 0.05 x 10(4) cells/cm(2). Thus, a high MSC inoculum density might be appropriate for the rapid differentiation of MSCs to chondrocytes.

Tan, S. L., et al. (2011). "Human amnion as a novel cell delivery vehicle for chondrogenic mesenchymal stem cells." Cell Tissue Bank **12**(1): 59-70.

This study investigates the feasibility of processed human amnion (HAM) as a substrate for chondrogenic differentiation of mesenchymal stem cells (MSCs). HAM preparations processed by air drying (AD) and freeze drying (FD) underwent histological examination and MSC seeding in chondrogenic medium for 15 days. Monolayer cultures were used as control for chondrogenic differentiation and HAMs without cell seeding were used as negative control. Qualitative observations were made using scanning electron microscopy analysis and quantitative analyses were based on the sulfated glycosaminoglycans (GAG) assays performed on day 1 and day 15. Histological examination of HAM substrates before seeding revealed a smooth surface in AD substrates, while the FD substrates exhibited a porous surface. Cell attachment to AD and FD substrates on day 15 was qualitatively comparable. GAG were significantly highly expressed in cells seeded on FD HAM substrates. This study indicates that processed HAM is a potentially valuable material as a cell-carrier for MSC differentiation.

Tanabe, S., et al. (2008). "Gene expression profiling of human mesenchymal stem cells for identification of novel markers in early- and late-stage cell culture." J Biochem **144**(3): 399-408.

Human mesenchymal stem cells (hMSCs) are multipotent cells that differentiate into several cell types, and are expected to be a useful tool for cellular therapy. Although the hMSCs differentiate into osteogenic cells during early to middle stages, this differentiation capacity decreases during the late stages of cell culture. To test a hypothesis that there are biomarkers indicating the differentiation potential of hMSCs, we performed microarray analyses and profiled the gene expression in six batches of hMSCs (passages 4-28). At least four genes [necdin homolog (mouse) (NDN), EPH receptor A5 (EPHA5), nephroblastoma overexpressed gene (NOV) and runt-related transcription factor 2 (RUNX2)] were identified correlating with the passage numbers in all six batches. The results showed that the osteogenic differentiation capacity of hMSCs is down-regulated in the late stages of cell culture. It seemed that adipogenic differentiation

capacity was also down-regulated in late stage of the culture. The cells in late stage are oligopotent and the genes identified in this study have the potential to act as quality-control markers of the osteogenic differentiation capacity of hMSCs.

Tang, K., et al. (2015). "Brief Report: Human Mesenchymal Stem-Like Cells Facilitate Floating Tumorigenic Cell Growth via Glutamine-Ammonium Cycle." Stem Cells **33**(9): 2877-2884.

How mesenchymal stem cells (MSCs) promote tumor growth remains incompletely understood. Here, we show that mesenchymal stem-like cells (MSLCs) are commonly present in malignant pleural effusion or ascites of cancer patients, where they directly interact with tumor cells. Chemokines and chemokine receptors, especially the CCL2/CCR2 pathway, are involved in this interaction. As a result, MSLCs exert tumor-promoting effects by enhancing the proliferation and colony formation of tumor-repopulating cells. The underlying molecular basis involves MSLC release of glutamine to tumorigenic cells. Inhibition of glutamine uptake impedes MSC-mediated tumor-promoting effects. More intriguingly, MSLCs take up tumor cell-released ammonium that, in turn, favors MSLC growth. Thus, glutamine and ammonium form a vicious cycle between MSLCs and tumorigenic cells. These findings suggest a potential clinical application by targeting MSLCs in patients with malignant pleural effusions or ascites.

Thankamony, S. P. and R. Sackstein (2011). "Enforced hematopoietic cell E- and L-selectin ligand (HCELL) expression primes transendothelial migration of human mesenchymal stem cells." Proc Natl Acad Sci U S A **108**(6): 2258-2263.

According to the multistep model of cell migration, chemokine receptor engagement (step 2) triggers conversion of rolling interactions (step 1) into firm adhesion (step 3), yielding transendothelial migration. We recently reported that glycosyltransferase-programmed stereosubstitution (GPS) of CD44 on human mesenchymal stem cells (hMSCs) creates the E-selectin ligand HCELL (hematopoietic cell E-selectin/L-selectin ligand) and, despite absence of CXCR4, systemically administered HCELL(+)hMSCs display robust osteotropism visualized by intravital microscopy. Here we performed studies to define the molecular effectors of this process. We observed that engagement of hMSC HCELL with E-selectin triggers VLA-4 adhesiveness, resulting in shear-resistant adhesion to ligand VCAM-1. This VLA-4 activation is mediated via a Rac1/Rap1 GTPase signaling pathway, resulting in transendothelial migration on stimulated human umbilical vein endothelial cells without chemokine input. These findings indicate that hMSCs coordinately integrate CD44 ligation and integrin activation, circumventing chemokine-mediated signaling, yielding a step 2-bypass pathway of the canonical multistep paradigm of cell migration.

Thapsukhon, B., et al. (2014). "Effect of topology of poly(L-lactide-co-epsilon-caprolactone) scaffolds on the response of cultured human umbilical cord Wharton's jelly-derived mesenchymal stem cells and neuroblastoma cell lines." J Biomater Sci Polym Ed **25**(10): 1028-1044.

In this study, for the first time, a biodegradable poly(L-lactide-co-epsilon-caprolactone), PLC 67:33 copolymer was developed for use as temporary scaffolds in reconstructive nerve surgery. The effect of the surface topology and pore architecture were studied on the biocompatibility for supporting the growth of human umbilical cord Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) and human neuroblastoma cells (hNBCs) as cell models. Porous PLC membranes were prepared by electrospinning and phase immersion precipitation with particulate leaching and nonporous PLC membranes were prepared by solvent casting. From the results, the porous PLC membranes can support hWJ-MSCs and hNBCs cells better than the nonporous PLC membrane, and the interconnected pore scaffold prepared by electrospinning exhibited a more significant supporting attachment of the cells than the open pore and nonporous membranes. We can consider that these electrospun PLC membranes with 3-D interconnecting fiber networks and a high porosity warrant a potential use as nerve guides in reconstructive nerve surgery.

Thiel, A., et al. (2015). "Human embryonic stem cell-derived mesenchymal cells preserve kidney function and extend lifespan in NZB/W F1 mouse model of lupus nephritis." Sci Rep **5**: 17685.

Adult tissue-derived mesenchymal stromal cells (MSCs) are showing promise in clinical trials for systemic lupus erythematosus (SLE). However, the inability to manufacture large quantities of functional cells from a single donor as well as donor-dependent variability in quality limits their clinical utility. Human embryonic stem cell (hESC)-derived MSCs are an alternative to adult MSCs that can circumvent issues regarding scalability and consistent quality due to their derivation from a renewable starting material. Here, we show that hESC-MSCs prevent the progression of fatal lupus nephritis (LN) in NZB/W F1 (BWF1) mice. Treatment led to statistically significant reductions in proteinuria and serum creatinine and preserved renal architecture. Specifically, hESC-MSC treatment prevented disease-associated interstitial inflammation, protein cast deposition, and infiltration of CD3(+)

lymphocytes in the kidneys. This therapy also led to significant reductions in serum levels of tumor necrosis factor alpha (TNFalpha) and interleukin 6 (IL-6), two inflammatory cytokines associated with SLE. Mechanistically, in vitro data support these findings, as co-culture of hESC-MSCs with lipopolysaccharide (LPS)-stimulated BWF1 lymphocytes decreased lymphocyte secretion of TNFalpha and IL-6, and enhanced the percentage of putative regulatory T cells. This study represents an important step in the development of a commercially scalable and efficacious cell therapy for SLE/LN.

Thimm, B. W., et al. (2011). "Initial cell pre-cultivation can maximize ECM mineralization by human mesenchymal stem cells on silk fibroin scaffolds." Acta Biomater **7**(5): 2218-2228.

Fast remineralization of bone defects by means of tissue engineering is one of many targets in orthopedic regeneration. This study investigated the influence of a range of pre-culture durations for human bone marrow derived mesenchymal stem cells (hMSC) before inducing differentiation into osteoblast-like cells. The aim was to find the conditions that lead to maximal extracellular matrix (ECM) mineralization, in terms of both amount and best distribution. Additionally, the influence of silk fibroin scaffold pore size on mineralization was assessed. The formation of mineralized ECM by hMSCs cultured in osteogenic medium on silk fibroin scaffolds was monitored and quantified for up to 72 days in culture using non-invasive time-lapse micro-computed tomography (micro-CT). ECM mineralization increased linearly 3 weeks after the beginning of the experiment with addition of differentiation medium. Biochemical end-point assays measured the amount of DNA, calcium deposits, alkaline phosphatase activity and cell metabolic activity to corroborate the hypothesis that an initial pre-culture period of hMSCs on silk fibroin scaffolds can accelerate mineralized ECM formation. According to the micro-CT analysis mineralization on silk fibroin scaffolds with pores of 112-224 mum diameter was most efficient with an initial cell pre-culture period of 9 days, showing 6.87+/-0.81x higher mineralization values during the whole cultivation period than without an initial cell pre-culture period.

Thomas, R. and E. Ratcliffe (2012). "Automated adherent human cell culture (mesenchymal stem cells)." Methods Mol Biol **806**: 393-406.

Human cell culture processes developed at research laboratory scale need to be translated to large-scale production processes to achieve commercial application to a large market. To allow this transition of scale with consistent process performance and control of costs, it will be necessary to reduce manual processing and increase automation. There are a number of commercially available platforms that will reduce manual process intervention and improve process control for different culture formats. However, in many human cell-based applications, there is currently a need to remain close to the development format, usually adherent culture on cell culture plastic or matrix-coated wells or flasks due to deterioration of cell quality in other environments, such as suspension. This chapter presents an example method for adherent automated human stem cell culture using a specific automated flask handling platform, the CompacT SelecT.

Thrivikraman, G., et al. (2014). "Intermittent electrical stimuli for guidance of human mesenchymal stem cell lineage commitment towards neural-like cells on electroconductive substrates." Biomaterials **35**(24): 6219-6235.

In the context of the role of multiple physical factors in dictating stem cell fate, the present paper demonstrates the effectiveness of the intermittently delivered external electric field stimulation towards switching the stem cell fate to specific lineage, when cultured in the absence of biochemical growth factors. In particular, our findings present the ability of human mesenchymal stem cells (hMSCs) to respond to the electric stimuli by adopting extended neural-like morphology on conducting polymeric substrates. Polyaniline (PANI) is selected as the model system to demonstrate this effect, as the electrical conductivity of the polymeric substrates can be systematically tailored over a broad range (10(-9) to 10 S/cm) from highly insulating to conducting by doping with varying concentrations (10(-5) to 1 m) of HCl. On the basis of the culture protocol involving the systematic delivery of intermittent electric field (dc) stimulation, the parametric window of substrate conductivity and electric field strength was established to promote significant morphological extensions, with minimal cellular damage. A time dependent morphological change in hMSCs with significant filopodial elongation was observed after 7 days of electrically stimulated culture. Concomitant with morphological changes, a commensurate increase in the expression of neural lineage commitment markers such as nestin and betaIII tubulin was recorded from hMSCs grown on highly conducting substrates, as revealed from the mRNA expression analysis using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) as well as by immune-fluorescence imaging. Therefore, the present work establishes the key role of intermittent and systematic delivery of electric stimuli as guidance cues in promoting neural-like differentiation of hMSCs, when grown on electroconductive substrates.

Tian, L. L., et al. (2011). "Human mesenchymal stem cells play a dual role on tumor cell growth in vitro and in vivo." J Cell Physiol **226**(7): 1860-1867.

Nowadays, some evidences demonstrate that human mesenchymal stem cells (hMSCs) favor tumor growth; however, others show that hMSCs can suppress tumorigenesis and tumor growth. With the indeterminateness of the effect of hMSCs on tumors, we investigated the effect of hMSCs on lung cancer cell line A549 and esophageal cancer cell line Eca-109 in vitro and in vivo. Our results revealed that hMSCs inhibited the proliferation and invasion of A549 and Eca-109 cells, arrested tumor cells in the G1 phase of the cell cycle and induced the apoptosis of tumor cells in vitro by using a co-culture system and the hMSCs-conditioned medium. However, animal study showed that hMSCs enhanced tumor formation and growth in vivo. Western blotting and immunoprecipitation data showed that the expressions of proliferating cell nuclear antigen (PCNA), Cyclin E, phospho-retinoblastoma protein (pRb), B-cell lymphoma/leukemia-2 (Bcl-2), Bcl-xL, and matrix metalloproteinase 2 (MMP-2) were downregulated and the formation of Cyclin E-cyclin-dependent kinase 2 (CDK2) complexes was inhibited in the tumor cells treated with the hMSCs-conditioned medium. According to the observation of tumor mass and the result of microvessel density (MVD), we found that the promoting role of hMSCs on tumor growth was related with the increase of tumor vessel formation. Our present study suggests that hMSCs have a contradictory effect on tumor cell growth between in vitro and in vivo, and therefore, the exploitation of hMSCs in new therapeutic strategies should be cautious under the malignant conditions.

Tierney, E. G., et al. (2013). "High levels of ephrinB2 over-expression increases the osteogenic differentiation of human mesenchymal stem cells and promotes enhanced cell mediated mineralisation in a polyethyleneimine-ephrinB2 gene-activated matrix." J Control Release **165**(3): 173-182.

Gene therapy can be combined with tissue engineering constructs to produce gene-activated matrices (GAMs) with enhanced capacity for repair. Polyethyleneimine (PEI), a non-viral vector, has previously been optimised for high efficiency gene transfer in rat mesenchymal stem cells (rMSCs). The use of PEI to transfect human MSCs (hMSCs) with ephrinB2 is assessed here. Recently a role for the ephrinB2 ligand and EphB4 receptor duo has been proposed in bone remodelling. Herein, over-expression of the ephrinB2 ligand resulted in increased osteogenic differentiation in hMSCs. As ephrinB2 is a cell surface anchored ligand which only interacts with cells expressing the cognate EphB4 receptor through direct contact, we have shown that direct cell-cell contact between two neighbouring cells is responsible for enhanced osteogenesis. In an effort to begin to elucidate the molecular mechanisms at play downstream of ephrinB2 over-expression, RT-PCR was performed on the GAMs which revealed no significant changes in runx2 or BMP2 expression but an upregulation of osterix (Osx) and Dlx5 expression prompting the belief that the mode of osteogenesis is independent of the BMP2 pathway. This select interaction, coupled with the transient gene expression profile of PEI, makes the PEI-ephrinB2 GAM an ideal candidate matrix for a bone targeted GAM.

Tobin, L. M., et al. (2013). "Human mesenchymal stem cells suppress donor CD4(+) T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease." Clin Exp Immunol **172**(2): 333-348.

Acute graft-versus-host disease (aGVHD) is a life-threatening complication following allogeneic haematopoietic stem cell transplantation (HSCT), occurring in up to 30-50% of patients who receive human leucocyte antigen (HLA)-matched sibling transplants. Current therapies for steroid refractory aGVHD are limited, with the prognosis of patients suboptimal. Mesenchymal stem or stromal cells (MSC), a heterogeneous cell population present in many tissues, display potent immunomodulatory abilities. Autologous and allogeneic ex-vivo expanded human MSC have been utilized to treat aGVHD with promising results, but the mechanisms of therapeutic action remain unclear. Here a robust humanized mouse model of aGVHD based on delivery of human peripheral blood mononuclear cells (PBMC) to non-obese diabetic (NOD)-severe combined immunodeficient (SCID) interleukin (IL)-2rgamma(null) (NSG) mice was developed that allowed the exploration of the role of MSC in cell therapy. MSC therapy resulted in the reduction of liver and gut pathology and significantly increased survival. Protection was dependent upon the timing of MSC therapy, with conventional MSC proving effective only after delayed administration. In contrast, interferon (IFN)-gamma-stimulated MSC were effective when delivered with PBMC. The beneficial effect of MSC therapy in this model was not due to the inhibition of donor PBMC chimerism, as CD45(+) and T cells engrafted successfully in this model. MSC therapy did not induce donor T cell anergy, FoxP3(+) T regulatory cells or cause PBMC apoptosis in this model; however, it was associated with the direct inhibition of donor CD4(+) T cell proliferation and reduction of human tumour necrosis factor-alpha in serum.

Tohamy, K. M., et al. (2018). "Novel alginate/hydroxyethyl cellulose/hydroxyapatite composite scaffold for bone regeneration: In vitro cell viability and proliferation of human mesenchymal stem cells." Int J Biol Macromol **112**: 448-460.

Sodium alginate (SA)/hydroxyethylcellulose (HEC)/hydroxyapatite (HA) composite scaffolds were explored for enhanced in vitro bone regeneration. The SA/HEC/HA composites were synthesized using the lyophilization technique and further cross-linked in the presence of calcium ions to form composite hydrogel networks. The physicochemical, thermal behavior and morphology properties of the prepared scaffolds were characterized through XRD, DSC/TGA, FTIR and SEM. Furthermore, the mechanical behavior of the under investigated scaffolds was determined using texture analyzer. The in vitro bioactivity in SBF and adsorption of bovine serum albumin as well as cell viability for all the prepared scaffolds were also tested. The results indicated that the higher HA concentration (40wt%) enhanced the mechanical properties (23.9MPa), bioactivity and protein adsorption. Cell viability of the tested scaffolds confirmed the non-toxicity of the fabricated systems on the human mesenchymal stem cells (hMSCs). Proliferation capability was also confirmed for the tested scaffolds after 3 and 7days, but the higher HA-containing scaffold showed increased cell populations specially after 7days compared to HA-free scaffolds. This novel composite material could be used in bone tissue engineering as a scaffold material to deliver cells and biologically active molecules.

Tokumitsu, A., et al. (2009). "Noninvasive estimation of cell cycle phase and proliferation rate of human mesenchymal stem cells by phase-shifting laser microscopy." Cytotechnology **59**(3): 161-167.

The simultaneous determination of the cell cycle phase of individual adherent mesenchymal stem cells (MSCs) using a fluorescence microscope after staining with 4',6-diamidine-2'-phenylindole dihydrochloride and bromodeoxyuridine and the laser phase shift by phase-shifting laser microscopy (PLM) revealed that the laser phase shift of cells in the G(2)/M phase was markedly higher than that of cells in the G(0)/G(1) phase. Even in the synchronous cultures to G(0)/G(1) and G(2)/M cell cycle phases, the laser phase shift of the cells in the G(2)/M phase was markedly higher than that of the cells in the G(0)/G(1) phase. The analysis of the cultures of MSCs from different donors with the addition of FGF2 at different concentrations revealed that there was a marked negative correlation between the average phase shift and mean generation time. In conclusion, it is possible to estimate noninvasively the proliferation activity of MSCs population by measuring the phase shift using PLM.

Tomar, G. B., et al. (2010). "Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine." Biochem Biophys Res Commun **393**(3): 377-383.

Mesenchymal stem cells (MSCs) are capable of self-renewal and differentiation into multiple cell lineages. Presently, bone marrow is considered as a prime source of MSCs; however, there are some drawbacks and limitations in use of these MSCs for cell therapy. In this study, we demonstrate that human gingival tissue-derived MSCs have several advantages over bone marrow-derived MSCs. Gingival MSCs are easy to isolate, homogenous and proliferate faster than bone marrow MSCs without any growth factor. Importantly, gingival MSCs display stable morphology and do not loose MSC characteristic at higher passages. In addition, gingival MSCs maintain normal karyotype and telomerase activity in long-term cultures, and are not tumorigenic. Thus, we reveal that human gingiva is a better source of MSCs than bone marrow, and large number of functionally competent clinical grade MSCs can be generated in short duration for cell therapy in regenerative medicine and tissue engineering.

Tome, M., et al. (2011). "miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells." Cell Death Differ **18**(6): 985-995.

In spite of the extensive potential of human mesenchymal stem cells (hMSCs) in cell therapy, little is known about the molecular mechanisms that regulate their therapeutic properties. We aimed to identify microRNAs (miRNAs) involved in controlling the transition between the resting and reparative phenotypes of hMSCs, hypothesizing that these miRNAs must be present in the undifferentiated cells and downregulated to allow initiation of distinct activation/differentiation programs. Differential miRNA expression analyses revealed that miR-335 is significantly downregulated upon hMSC differentiation. In addition, hMSCs derived from a variety of tissues express miR-335 at a higher level than human skin fibroblasts, and overexpression of miR-335 in hMSCs inhibited their proliferation and migration, as well as their osteogenic and adipogenic potential. Expression of miR-335 in hMSCs was upregulated by the canonical Wnt signaling pathway, a positive regulator of MSC self-renewal, and downregulated by interferon-gamma (IFN-gamma), a pro-inflammatory cytokine that has an important role in activating the immunomodulatory properties of hMSCs. Differential gene expression analyses, in combination with

computational searches, defined a cluster of 62 putative target genes for miR-335 in hMSCs. Western blot and 3'UTR reporter assays confirmed RUNX2 as a direct target of miR-335 in hMSCs. These results strongly suggest that miR-335 downregulation is critical for the acquisition of reparative MSC phenotypes.

Tremain, N., et al. (2001). "MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages." Stem Cells **19**(5): 408-418.

Mesenchymal stem cells (MSCs) isolated from the bone marrow of adult organisms are capable of differentiating into adipocytes, chondrocytes, myoblasts, osteoblasts, and hematopoiesis-supporting stroma. We recently demonstrated that MSCs also adopt glial cell fates when transplanted into the developing central nervous system and hence can produce tissue elements derived from a separate embryonic layer. Despite these remarkable properties, it has been difficult to establish specific criteria to characterize MSCs. Using a modified protocol for micro-serial analysis of gene expression, we cataloged 2,353 unique genes expressed by a single cell-derived colony of undifferentiated human MSCs. This analysis revealed that the MSC colony simultaneously expressed transcripts characteristic of various mesenchymal cell lineages including chondrocytes, myoblasts, osteoblasts, and hematopoiesis-supporting stroma. Therefore, the profile of expressed transcripts reflects the developmental potential of the cells. Additionally, the MSC colony expressed mRNAs characteristic of endothelial, epithelial and neuronal cell lineages, a combination that provides a unique molecular signature for the cells. Other expressed transcripts included various products involved in wound repair as well as several neurotrophic factors. A total of 268 novel transcripts were also identified, one of which is the most abundantly expressed mRNA in MSCs. This study represents the first extensive gene expression analysis of MSCs and as such reveals new insight into the biology, ontogeny, and in vivo function of the cells.

Trivanovic, D., et al. (2014). "Characteristics of human adipose mesenchymal stem cells isolated from healthy and cancer affected people and their interactions with human breast cancer cell line MCF-7 in vitro." Cell Biol Int **38**(2): 254-265.

Adipose tissue is an attractive source of mesenchymal stem/stromal cells (MSCs) with potential applications in reconstructive plastic surgery and regenerative medicine. The aim of this study was to characterise human adipose tissue MSCs (ASCs) derived from healthy individuals and cancer patients and to compare their interactions with tumour cells. ASCs were isolated from adipose tissue of healthy donors, breast cancer-adjacent adipose tissue of breast cancer patients and tumour-adjacent adipose tissue of non-breast cancer patients. Their proliferation, differentiation, immunophenotype and gene expression were assessed and effects on the proliferation of human breast cancer cell line MCF-7 compared. ASCs from all sources exhibited similar morphology, proliferative and differentiation potential, showing the characteristic pattern of mesenchymal surface markers expression (CD90, CD105, CD44H, CD73) and the lack of HLA-DR and hematopoietic markers (CD11a, CD33, CD45, Glycophorin-CD235a), but uneven expression of CD34. ASCs also shared a common positive gene expression of HLA-DR, HLA-A, IL-6, TGF-beta and HIF-1, but were negative for HLA-G, while the expression levels of Cox-2 and IDO-1 varied. All ASCs significantly stimulated the proliferation of MCF-7 tumour cells in direct mixed co-cultures and transwell system, although their conditioned media displayed antiproliferative activity. Data obtained showed that ASCs with similar characteristics are easily isolated from various donors and sites of origin, although ASCs could both suppress and favour tumour cells growth, emphasising the importance of cellular context within the microenvironment and pointing to the significance of safety studies to exclude any potential clinical risk of their application in regenerative medicine.

Trivedi, H. L., et al. (2008). "Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin." Transplant Proc **40**(4): 1135-1139.

BACKGROUND: Type 1 diabetes mellitus (DM) is an autoimmune disorder with disturbed glucose/insulin metabolism, which has no medical treatment other than life-long insulin therapy, despite which 30% of subjects develop organ failure. Herein we have reported the use of human adipose-tissue-derived, insulin-making mesenchymal stem cells (h-AD-MSC) transfused with unfractionated cultured bone marrow (CBM) in 5 insulinopenic DM patients. PATIENTS AND METHODS: Five (M:F, 2:3) insulinopenic DM patients of 0.6 to 10 years' duration, ages 14 to 28 years under treatment insulin (Human with 14-70 U/d) showed postprandial blood sugars between 156 to 470 mg%, glycosylated hemoglobin 6.8% to 9.9% and c-peptide levels of 0.02 to 0.2 ng/mL. They underwent intraportal administration of xenogeneic-free h-AD-MSC (mean dose = 1.5 mL; cell counts, 2.1 x 10(3)/muL). The CD45-/90+/73(+) cells (29.8/16.8%) showed c-peptide levels of 3.08 ng/mL, insulin level of 1578 micro IU/mL. The aliquot was supplemented with CBM (mean dose 94 mL with cell counts: 18.7 x 10(3)/microL) containing CD45-/34+

elements of 0.93%. The Institutional Review Board approved the study protocol and consent forms. RESULTS: All patients were successfully infused CBM plus h-AD-MSC without any untoward effects and showed 30% to 50% decreased insulin requirements with 4- to 26-fold increased serum c-peptide levels, with a mean follow-up of 2.9 months. CONCLUSION: This report describes safe and effective treatment of insulinopenic diabetics using insulin-producing h-AD-MSC plus CBM without xenogeneic materials.

Trubiani, O., et al. (2012). "Overexpression of interleukin-6 and -8, cell growth inhibition and morphological changes in 2-hydroxyethyl methacrylate-treated human dental pulp mesenchymal stem cells." Int Endod J **45**(1): 19-25.

AIM: To evaluate morphological features, cell growth and interleukin-6 (IL-6) and interleukin-8 (IL-8) secretion in expanded ex vivo human dental pulp mesenchymal stem cells (DP-MSCs) after exposure to 2-hydroxyethyl methacrylate (HEMA). METHODOLOGY: Dental pulp mesenchymal stem cells were derived from the dental pulps of 10 young donors. After in vitro isolation, DP-MSCs were treated with 3 and 5 mmol L(-1) HEMA, and after 24, 48 and 72 h of incubation, their morphological features, cell growth, IL-6 and IL-8 secretion were analysed. Differences in the cell growth and in the interleukin secretion were analysed for statistical significance with two-way anova tests and the Holm-Sidak method for multiple comparisons. RESULTS: Dental pulp mesenchymal stem cells revealed a decrease in cell growth with both treatments ($P < 0.05$), more evident at 5 mmol L(-1) . Microscopic analysis displayed extensive cytotoxic effects in treated cells, which lost their fibroblastoid features and became retracted, even roundish, with a large number of granules. An up-regulation of IL-6 and IL-8 in treated cells cytokines was evident ($P < 0.05$). CONCLUSIONS: 2-Hydroxyethyl methacrylate exhibited cytotoxicity, inhibited cell growth and induced morphological changes in cultured DP-MSCs. Moreover, in treated samples, an up-regulation of soluble mediators of inflammation such as IL-6 and IL-8 cytokines was found. The direct application of HEMA potentially induces an inflammation process that could be the starting point for toxic response and cell damage in DP-MSCs.

Varga, N., et al. (2011). "Mesenchymal stem cell like (MSCl) cells generated from human embryonic stem cells support pluripotent cell growth." Biochem Biophys Res Commun **414**(3): 474-480.

Mesenchymal stem cell like (MSCl) cells were generated from human embryonic stem cells (hESC) through embryoid body formation, and isolated by adherence to plastic surface. MSCl cell lines could be propagated without changes in morphological or functional characteristics for more than 15 passages. These cells, as well as their fluorescent protein expressing stable derivatives, efficiently supported the growth of undifferentiated human embryonic stem cells as feeder cells. The MSCl cells did not express the embryonic (Oct4, Nanog, ABCG2, PODXL, or SSEA4), or hematopoietic (CD34, CD45, CD14, CD133, HLA-DR) stem cell markers, while were positive for the characteristic cell surface markers of MSCs (CD44, CD73, CD90, CD105). MSCl cells could be differentiated toward osteogenic, chondrogenic or adipogenic directions and exhibited significant inhibition of mitogen-activated lymphocyte proliferation, and thus presented immunosuppressive features. We suggest that cultured MSCl cells can properly model human MSCs and be applied as efficient feeders in hESC cultures.

Vellasamy, S., et al. (2013). "Mesenchymal stem cells of human placenta and umbilical cord suppress T-cell proliferation at G0 phase of cell cycle." Cell Biol Int **37**(3): 250-256.

Mesenchymal stem cells (MSC) generated from human umbilical cord (UC-MSC) and placenta (PLC-MSC) were assessed and compared for their immunomodulatory function on T cells proliferation by analysis of the cell cycle. Mitogen stimulated or resting T cells were co-cultured in the presence or absence of MSC. T-cell proliferation was assessed by tritiated thymidine ((3) H-TdR) assay and the mechanism of inhibition was examined bycell cycle and apoptosis assay. Both UC-MSC and PLC-MSC profoundly inhibited the proliferation of T-cell, mainly via cell-to-cell contact. MSC-mediated anti-proliferation does not lead to apoptosis,but prevented T cells from entering S phase and they therefore accumulated in the G(0) /G(1) phases. The anti-proliferative activity of MSC was related to this cell cycle arrest of T-cell. UC-MSC produced a greater inhibition than PLC-MSC in all measured parameters.

Venugopal, B., et al. (2018). "Cell density overrides the effect of substrate stiffness on human mesenchymal stem cells' morphology and proliferation." Biomater Sci **6**(5): 1109-1119.

The effect of substrate stiffness on the cellular morphology, proliferation, and differentiation of human mesenchymal stem cells (hMSCs) has been extensively researched and well established. However, the majority of these studies are done with a low seeding density where cell to cell interactions do not play a significant role. While these conditions permit an analysis of cell-substratum interactions at the single

cell level, such a model system fails to capture a critical aspect of the cellular micro-environment in vivo, i.e. the cell-cell interaction via matrix deformation (i.e., strain). To address this question, we seeded hMSCs on soft poly-acrylamide (PAA) gels, at a seeding density that permits cells to be mechanically interacting via the underlying substrate. We found that as the intercellular distance decreases with the increasing seeding density, cellular sensitivity towards the substrate rigidity becomes significantly diminished. With the increasing seeding density, the cell spread area increased on a soft substrate (500 Pa) but reduced on an even slightly stiffer substrate (2 kPa) as well as on glass making them indistinguishable at a high seeding density. Not only in terms of cell spread area but also at a high seeding density, cells formed mature focal adhesions and prominent stress fibres on a soft substrate similar to that of the cells being cultured on a stiff substrate. The decreased intercellular distance also influenced the proliferation rate of the cells: higher seeding density on the soft substrate showed cell cycle progression similar to that of the cells on glass substrates. In summary, this paper demonstrates how the effect of substrate rigidity on the cell morphology and fate is a function of inter-cellular distance when seeded on a soft substrate. Our AFM data suggest that such changes happen due to local strain stiffening of the soft PAA gel, an effect that has been rarely reported in the literature so far.

Veraitch, O., et al. (2017). "Induction of hair follicle dermal papilla cell properties in human induced pluripotent stem cell-derived multipotent LNGFR(+)THY-1(+) mesenchymal cells." Sci Rep **7**: 42777.

The dermal papilla (DP) is a specialised mesenchymal component of the hair follicle (HF) that plays key roles in HF morphogenesis and regeneration. Current technical difficulties in preparing trichogenic human DP cells could be overcome by the use of highly proliferative and plastic human induced pluripotent stem cells (hiPSCs). In this study, hiPSCs were differentiated into induced mesenchymal cells (iMCs) with a bone marrow stromal cell phenotype. A highly proliferative and plastic LNGFR(+)THY-1(+) subset of iMCs was subsequently programmed using retinoic acid and DP cell activating culture medium to acquire DP properties. The resultant cells (induced DP-substituting cells [iDPSCs]) exhibited up-regulated DP markers, interacted with human keratinocytes to up-regulate HF related genes, and when co-grafted with human keratinocytes in vivo gave rise to fibre structures with a hair cuticle-like coat resembling the hair shaft, as confirmed by scanning electron microscope analysis. Furthermore, iDPSCs responded to the clinically used hair growth reagent, minoxidil sulfate, to up-regulate DP genes, further supporting that they were capable of, at least in part, reproducing DP properties. Thus, LNGFR(+)THY-1(+) iMCs may provide material for HF bioengineering and drug screening for hair diseases.

Veriter, S., et al. (2015). "Human Adipose-Derived Mesenchymal Stem Cells in Cell Therapy: Safety and Feasibility in Different "Hospital Exemption" Clinical Applications." PLoS One **10**(10): e0139566.

Based on immunomodulatory, osteogenic, and pro-angiogenic properties of adipose-derived stem cells (ASCs), this study aims to assess the safety and efficacy of ASC-derived cell therapies for clinical indications. Two autologous ASC-derived products were proposed to 17 patients who had not experienced any success with conventional therapies: (1) a scaffold-free osteogenic three-dimensional graft for the treatment of bone non-union and (2) a biological dressing for dermal reconstruction of non-healing chronic wounds. Safety was studied using the quality control of the final product (genetic stability, microbiological/mycoplasma/endotoxin contamination) and the in vivo evaluation of adverse events after transplantation. Feasibility was assessed by the ability to reproducibly obtain the final ASC-based product with specific characteristics, the time necessary for graft manufacturing, the capacity to produce enough material to treat the lesion, the surgical handling of the graft, and the ability to manufacture the graft in line with hospital exemption regulations. For 16 patients (one patient did not undergo grafting because of spontaneous bone healing), in-process controls found no microbiological/mycoplasma/endotoxin contamination, no obvious deleterious genomic anomalies, and optimal ASC purity. Each type of graft was reproducibly obtained without significant delay for implantation and surgical handling was always according to the surgical procedure and the implantation site. No serious adverse events were noted for up to 54 months. We demonstrated that autologous ASC transplantation can be considered a safe and feasible therapy tool for extreme clinical indications of ASC properties and physiopathology of disease.

Visciano, C., et al. (2015). "Mast cells induce epithelial-to-mesenchymal transition and stem cell features in human thyroid cancer cells through an IL-8-Akt-Slug pathway." Oncogene **34**(40): 5175-5186.

There is increasing evidence that mast cells (MCs) and their mediators are involved in the remodeling of the tumor microenvironment and promote tumor growth, angiogenesis and metastasis. We have found that an increased density of MCs in thyroid cancer (TC) correlates with enhanced invasiveness. However, the MC-derived factors

responsible for this activity and the mechanisms by which they enhance TC invasiveness remain unidentified. Here, we report that MCs, when activated by TC cells, produce soluble factors that induce epithelial-to-mesenchymal transition (EMT) and stemness features of TC cells. We identified CXCL8/interleukin (IL)-8 as the main mediator contained in activated MC conditioned media (CM) capable of inducing both EMT and stemness of TC cells. Mechanistically, MC CM or exogenous IL-8 stimulated Akt phosphorylation and Slug expression in TC cells. The inhibition of the Akt pathway or depletion of the Slug transcription factor by RNA interference, reverted EMT and stemness responses. TC cells stably transfected with exogenous IL-8 underwent EMT, displayed increased stemness and enhanced tumorigenicity with respect to control cells. The analysis of TC surgical specimens by immunohistochemical analysis demonstrated a positive correlation between MC density (Tryptase(+) cells) and stemness features (OCT4 staining). Taken together, our data identify an MC-dependent IL-8-Akt-Slug pathway that sustains EMT/stemness of TC cells. The blockade of this circuit might be exploited for the therapy of advanced TC.

Wagner, W., et al. (2007). "Adhesion of hematopoietic progenitor cells to human mesenchymal stem cells as a model for cell-cell interaction." Exp Hematol **35**(2): 314-325.

OBJECTIVE: The significant role of direct contact between hematopoietic progenitor cells (HPC) and the cellular microenvironment for maintaining "stemness" has been demonstrated. Human mesenchymal stem cell (MSC) feeder layers represent a surrogate model for this interaction. Specific adhesion molecules are responsible for this cell-cell contact. METHODS: To define cell-cell contact between HPC and MSC, we have studied adhesive interaction of various fractions of HPC by using a novel assay based on gravitational force upon inversion. Adherent and nonadherent cells were separated and further analyzed with regard to gene expression and long-term hematopoietic culture initiating cell (LTC-IC) frequency. RESULTS: HPC subsets with higher self-renewing capacity demonstrated significantly higher adherence to human MSC (CD34(+) vs CD34(-), CD34(+)/CD38(-) vs CD34(+)/CD38(+), slow dividing fraction vs fast dividing fraction). LTC-IC frequency was significantly higher in the adherent fraction than in the nonadherent fraction. Furthermore, genes coding for adhesion proteins and extracellular matrix were higher expressed in the adherent subsets of CD34(+) cells (fibronectin 1, cadherin 11, vascular cell adhesion molecule-1, connexin 43, integrin beta-like 1, and TGFBI). CONCLUSION: In this study we have demonstrated that primitive subsets of HPC have higher affinity to human MSC. The essential role of specific junction proteins for stabilization of cell-cell contact is indicated by their significant higher expression.

Wakao, S., et al. (2012). "Isolation of adult human pluripotent stem cells from mesenchymal cell populations and their application to liver damages." Methods Mol Biol **826**: 89-102.

We have found a novel type of pluripotent stem cells, Multilineage-differentiating stress enduring (Muse) cells that can be isolated from mesenchymal cell populations. Muse cells are characterized by stress tolerance, expression of pluripotency markers, self-renewal, and the ability to differentiate into endodermal-, mesodermal-, and ectodermal-lineage cells from a single cell, demonstrating that they are pluripotent stem cells. They can be isolated as cells positive for stage-specific embryonic antigen-3, a human pluripotent stem cell marker. Here, we introduce the isolation method for Muse cells and the effect of transplantation of these cells on chronic liver diseases.

Walter, M. N., et al. (2015). "Human mesenchymal stem cells stimulate EaHy926 endothelial cell migration: combined proteomic and in vitro analysis of the influence of donor-donor variability." J Stem Cells Regen Med **11**(1): 18-24.

Mesenchymal stem cells (MSCs) stimulate angiogenesis within a wound environment and this effect is mediated through paracrine interactions with the endothelial cells present. Here we report that human MSC-conditioned medium (n=3 donors) significantly increased EaHy-926 endothelial cell adhesion and cell migration, but that this stimulatory effect was markedly donor-dependent. MALDI-TOF/TOF mass spectrometry demonstrated that whilst collagen type I and fibronectin were secreted by all of the MSC cultures, the small leucine rich proteoglycan, decorin was secreted only by the MSC culture that was least effective upon EaHy-926 cells. These individual extracellular matrix components were then tested as culture substrata. EaHy-926 cell adherence was greatest on fibronectin-coated surfaces with least adherence on decorin-coated surfaces. Scratch wound assays were used to examine cell migration. EaHy-926 cell scratch wound closure was quickest on substrates of fibronectin and slowest on decorin. However, EaHy-926 cell migration was stimulated by the addition of MSC-conditioned medium irrespective of the types of culture substrates. These data suggest that whilst the MSC secretome may generally be considered angiogenic, the composition of the secretome is variable and this variation probably contributes to

donor-donor differences in activity. Hence, screening and optimizing MSC secretomes will improve the clinical effectiveness of pro-angiogenic MSC-based therapies.

Wan, C., et al. (2006). "Nonadherent cell population of human marrow culture is a complementary source of mesenchymal stem cells (MSCs)." J Orthop Res **24**(1): 21-28.

To obtain enough quantity of osteogenic cells is a challenge for successful cell therapy in bone defect treatment, and cell numbers were usually achieved by culturing bone marrow cells in a relatively long duration. This study reports a simple and cost-effective method to enhance the number of mesenchymal stem cells (MSCs) by collecting and replating the nonadherent cell population of marrow MSCs culture. Bone marrow MSCs were isolated from 11 patients, cultured at a density of 1 x 10(5)/cm(2) to 1 x 10(6)/cm(2) in flasks. For the first three times of media change, the floating cells were centrifuged and replated in separate flasks. The total number of cells in both the primary and replating flasks were counted at day 21. Cell proliferation rate, potentials for osteogenic, chondrognenic, and adipogenic differentiation were examined in both cell types in vitro. In vivo osteogenic potentials of the cells were also tested in mice implantation model. The results showed that MSCs derived from nonadherent cell population of marrow cell cultures have similar cell proliferation and differentiation potentials as the originally attached MSCs in vitro. When implanted with hydroxyapatite/tricalcium phosphate (HA-TCP) materials subcutaneously in serve combined immune deficiency (SCID) mice, newly formed bony tissues were found in both cell type groups with osteocalcin expression. We have obtained 36.6% (20.70%-44.97%) more MSCs in the same culture period when the nonadherent cell populations were collected. The findings confirmed that the nonadherent cell population in the bone marrow culture is a complementary source of MSCs, collecting these cells is a simple and cost-effective way to increase MSCs numbers and reduce the time required for culturing MSCs for clinical applications.

Wang, F. Z., et al. (2008). "Promotion of cell proliferation by HBXIP via upregulation of human telomerase reverse transcriptase in human mesenchymal stem cells." Acta Pharmacol Sin **29**(1): 83-89.

AIM: We previously found that the hepatitis B X-interacting protein (HBXIP) was able to promote the proliferation of cells. Telomerase activity is known to be critical in cellular senescence and its level is modulated by the regulation of the telomerase catalytic subunit, telomerase reverse transcriptase (TERT), at both the transcriptional and post-transcriptional levels. To investigate the mechanism of promoting proliferation by HBXIP, the effect of HBXIP on human TERT (hTERT) was investigated in human mesenchymal stem cells (hMSC). METHODS: BMMS-03 cells and hMSC from the bone marrow of a 4-month-old elicited fetus, were transiently transfected with the pcDNA3-hbxip plasmid encoding the HBXIP gene and pSilencer-hbxip plasmid encoding RNA interference (RNAi) targeting HBXIP mRNA, followed by the examination of the hTERT promoter reporter gene by luciferase assay, and the detection of telomerase activity by telomeric repeat amplication protocol, respectively, as well as the expression levels of hTERT, c-Myc, and Bcl-2 by Western blot analysis. RESULTS: The overexpression of HBXIP led to a significant upregulation of hTERT promoter activity, telomerase activity, and the expression levels of hTERT, c-Myc, and Bcl-2 in BMMS-03 cells. RNAi targeting HBXIP mRNA produced the opposite results completely. CONCLUSION: Our data demonstrated that HBXIP significantly stimulated the transcription and expression of hTERT and increased the activity of telomerase in BMMS-03 cells, which provides a new insight into the mechanism of promoting cell proliferation by HBXIP.

Xue, R., et al. (2013). "Effects of matrix elasticity and cell density on human mesenchymal stem cells differentiation." J Orthop Res **31**(9): 1360-1365.

Human mesenchymal stem cells (hMSCs) can differentiate into various cell types, including osteogenic and chondrogenic cells. The matrix elasticity and cell seeding density are important factors in hMSCs differentiation. We cultured hMSCs at different seeding densities on polyacrylamide hydrogels with different stiffness corresponding to Young's moduli of 1.6 +/- 0.3 and 40 +/- 3.6 kPa. The promotion of osteogenic marker expression by hard gel is overridden by a high seeding density. Cell seeding density, however, did not influence the chondrogenic marker expressions induced by soft gel. These findings suggest that interplays between cell-matrix and cell-cell interactions contribute to hMSCs differentiation. The promotion of osteogenic differentiation on hard matrix was shown to be mediated through the Ras pathway. Inhibition of Ras (RasN17) significantly decreased ERK, Smad1/5/8 and AKT activation, and osteogenic markers expression. However, constitutively active Ras (RasV12) had little effect on osteogenic marker expression, suggesting that the Ras pathways are necessary but not sufficient for osteogenesis. Taken together, our results indicate that matrix elasticity and cell density are important microenvironmental cues driving hMSCs proliferation and differentiation.

Yamada, Y., et al. (2006). "Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy." Biomaterials **27**(20): 3766-3781.

We investigated gene expression patterns and functional classifications regarding the clusters of human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs)--which possess a multipotent ability--because little is known about the precise moleculobiological clues by which these cells activate their differentiating ability or functionality to eventually form dentin and bone, respectively. We first verified the expressions of the alkaline phosphatase (ALP) gene, dentin matrix protein 1 (DMP-1), and dentinsialophosphoprotein (DSPP) by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and consequently discovered the high expressions of these genes. Total RNA was also followed by hybridization with a human microarray system consisting of 12,814 genes. Analyses of gene expression patterns indicated several genes which encode extracellular matrix components, cell adhesion molecules, growth factors, and transcription regulators. Functional and clustering analyses of differences in gene expression levels revealed cell signaling, cell communication, or cell metabolism. In the future, information on the gene expression patterns of hDPSCs and hMSCs might be useful in determining the detailed functional roles of the relevant genes and applicable to stem cell therapies, and these cells could also be used as multipotent cell sources for gene technology and tissue engineering technology.

Yamaguchi, S., et al. (2018). "Age of donor of human mesenchymal stem cells affects structural and functional recovery after cell therapy following ischaemic stroke." J Cereb Blood Flow Metab **38**(7): 1199-1212.

Cell transplantation therapy offers great potential to improve impairments after stroke. However, the importance of donor age on therapeutic efficacy is unclear. We investigated the regenerative capacity of transplanted cells focusing on donor age (young vs. old) for ischaemic stroke. The quantities of human mesenchymal stem cell (hMSC) secreted brain-derived neurotrophic factor in vitro and of monocyte chemotactic protein-1 at day 7 in vivo were both significantly higher for young hMSC compared with old hMSC. Male Sprague-Dawley rats subjected to transient middle cerebral artery occlusion that received young hMSC (trans-arterially at 24 h after stroke) showed better behavioural recovery with prevention of brain atrophy compared with rats that received old hMSC. Histological analysis of the peri-infarct cortex showed that rats treated with young hMSC had significantly fewer microglia and more vessels covered with pericytes. Interestingly, migration of neural stem/progenitor cells expressing Musashi-1 positively correlated with astrocyte process alignment, which was more pronounced for young hMSC. Aging of hMSC may be a critical factor that affects cell therapy outcomes, and transplantation of young hMSC appears to provide better functional recovery through anti-inflammatory effects, vessel maturation, and neurogenesis potentially by the dominance of trophic factor secretion.

Yang, C., et al. (2014). "Conditioned media from human adipose tissue-derived mesenchymal stem cells and umbilical cord-derived mesenchymal stem cells efficiently induced the apoptosis and differentiation in human glioma cell lines in vitro." Biomed Res Int **2014**: 109389.

Human mesenchymal stem cells (MSCs) have an intrinsic property for homing towards tumor sites and can be used as tumor-tropic vectors for tumor therapy. But very limited studies investigated the antitumor properties of MSCs themselves. In this study we investigated the antiglioma properties of two easily accessible MSCs, namely, human adipose tissue-derived mesenchymal stem cells (ASCs) and umbilical cord-derived mesenchymal stem cells (UC-MSCs). We found (1) MSC conditioned media can significantly inhibit the growth of human U251 glioma cell line; (2) MSC conditioned media can significantly induce apoptosis in human U251 cell line; (3) real-time PCR experiments showed significant upregulation of apoptotic genes of both caspase-3 and caspase-9 and significant downregulation of antiapoptotic genes such as survivin and XIAP after MSC conditioned media induction in U 251 cells; (4) furthermore, MSCs conditioned media culture induced rapid and complete differentiation in U251 cells. These results indicate MSCs can efficiently induce both apoptosis and differentiation in U251 human glioma cell line. Whereas UC-MSCs are more efficient for apoptosis induction than ASCs, their capability of differentiation induction is not distinguishable from each other. Our findings suggest MSCs themselves have favorable antitumor characteristics and should be further explored in future glioma therapy.

Yao, J., et al. (2012). "Tissue inhibitor of matrix metalloproteinase-3 or vascular endothelial growth factor transfection of aged human mesenchymal stem cells enhances cell therapy after myocardial infarction." Rejuvenation Res **15**(5): 495-506.

Mesenchymal stem cell (MSC) transplantation has been proposed as a potential therapeutic approach

for ischemic heart disease, but the regenerative capacity of these cells decreases with age. In this study, we genetically engineered old human MSCs (O-hMSCs) with tissue inhibitor of matrix metalloproteinase-3 (TIMP3) and vascular endothelial growth factor (VEGF) and evaluated the effects on the efficacy of cell-based gene therapy in a rat myocardial infarction (MI) model. Cultured O-hMSCs were transfected with TIMP3 (O-TIMP3) or VEGF (O-VEGF) and compared with young hMSCs (Y-hMSCs) and non-transfected O-hMSCs for growth, clonogenic capacity, and differentiation potential. In vivo, rats were subjected to left coronary artery ligation with subsequent injection of Y-hMSCs, O-hMSCs, O-TIMP3, O-VEGF, or medium. Echocardiography was performed prior to and at 1, 2, and 4 weeks after MI. Myocardial levels of matrix metalloproteinase-2 (MMP2), MMP9, TIMP3, and VEGF were assessed at 1 week. Hemodynamics, morphology, and histology were measured at 4 weeks. In vitro, genetically modified O-hMSCs showed no changes in growth, colony formation, or multi-differentiation capacity. In vivo, transplantation with O-TIMP3, O-VEGF, or Y-hMSCs increased capillary density, preserved cardiac function, and reduced infarct size compared to O-hMSCs and medium control. O-TIMP3 and O-VEGF transplantation enhanced TIMP3 and VEGF expression, respectively, in the treated animals. O-hMSCs genetically modified with TIMP3 or VEGF can increase angiogenesis, prevent adverse matrix remodeling, and restore cardiac function to a degree similar to Y-hMSCs. This gene-modified cell therapy strategy may be a promising clinical treatment to rejuvenate stem cells in elderly patients.

Yen, B. L., et al. (2009). "Brief report--human embryonic stem cell-derived mesenchymal progenitors possess strong immunosuppressive effects toward natural killer cells as well as T lymphocytes." Stem Cells **27**(2): 451-456.

The derivation of mesenchymal progenitors from human embryonic stem cells (hESCs) has recently been reported. We studied the immune characteristics of these hESC-derived mesenchymal progenitors (EMPs) and their interactions with T lymphocytes and natural killer cells (NKs), two populations of lymphocytes with important roles in transplantation immunology. EMPs express a number of bone marrow mesenchymal stromal cell (BMMSC) markers, as well as the hESC marker SSEA-4. Immunologically, EMPs do not express HLA-DR or costimulatory molecules. On the other hand, HLA-G, a nonclassic MHC I protein involved in mediating maternal-fetal tolerance, can be found on the surface of EMPs, and its expression is increased after interferon-gamma stimulation. EMPs can suppress CD4(+) or CD8(+) lymphocyte proliferation, similar to BMMSCs. However, EMPs are more resistant to NK-mediated lysis than BMMSCs and can suppress the cytotoxic effects of activated NKs, as well as downregulating the NK-activating receptors NKp30 and NKp46. With their broad immunosuppressive properties, EMPs may represent a new potential cell source for therapeutic use.

Yiang, G. T., et al. (2015). "Acetaminophen induces JNK/p38 signaling and activates the caspase-9-3-dependent cell death pathway in human mesenchymal stem cells." Int J Mol Med **36**(2): 485-492.

Acetaminophen (APAP) is a widely used analgesic and antipyretic drug. Generally, the therapeutic dose of APAP is clinically safe, however, high doses of APAP can cause acute liver and kidney injury. Therefore, the majority of previous studies have focussed on elucidating the mechanisms of APAP-induced hepatotoxicity and nephrotoxicity, in addition to examining ways to treat these conditions in clinical cases. However, few studies have reported APAP-induced intoxication in human stem cells. Stem cells are important in cell proliferation, differentiation and repair during human development, particularly during fetal and child development. At present, whether APAP causes cytotoxic effects in human stem cells remains to be elucidated, therefore, the present study aimed to investigate the cellular effects of APAP treatment in human stem cells. The results of the present study revealed that high-dose APAP induced more marked cytotoxic effects in human mesenchymal stem cells (hMSCs) than in renal tubular cells. In addition, increased levels of hydrogen peroxide (H2O2), phosphorylation of c-Jun N-terminal kinase and p38, and activation of caspase-9/-3 cascade were observed in the APAP-treated hMSCs. By contrast, antioxidants, including vitamin C reduced APAP-induced augmentations in H2O2 levels, but did not inhibit the APAP-induced cytotoxic effects in the hMSCs. These results suggested that high doses of APAP may cause serious damage towards hMSCs.

Yokokura, T., et al. (2017). "The Short-Stature Homeobox-Containing Gene (shox/SHOX) Is Required for the Regulation of Cell Proliferation and Bone Differentiation in Zebrafish Embryo and Human Mesenchymal Stem Cells." Front Endocrinol (Lausanne) **8**: 125.

The short-stature homeobox-containing gene (SHOX) was originally discovered as one of genes responsible for idiopathic short-stature syndromes in humans. Previous studies in animal models have shown the evolutionarily conserved link between this gene and skeletal formation in early embryogenesis. Here, we characterized developmental roles of shox/SHOX in zebrafish embryos and human mesenchymal stem cells (hMSCs) using loss-of-function approaches.

Morpholino oligo-mediated knockdown of zebrafish shox markedly hindered cell proliferation in the anterior region of the pharyngula embryos, which was accompanied by reduction in the dlx2 expression at mesenchymal core sites for future pharyngeal bones. In addition, the impaired shox expression transiently increased expression levels of skeletal differentiation genes in early larval stage. In cell culture studies, we found that hMSCs expressed SHOX; the siRNA-mediated blockade of SHOX expression significantly blunted cell proliferation in undifferentiated hMSCs but the loss of SHOX expression did augment the expressions of subsets of early osteogenic genes during early osteoblast differentiation. These data suggest that shox/SHOX maintains the population of embryonic bone progenitor cells by keeping its proliferative status and by repressing the onset of early osteogenic gene expression. The current study for the first time shows cellular and developmental responses caused by shox/SHOX deficiency in zebrafish embryos and hMSCs, and it expands our understanding of the role of this gene in early stages of skeletal growth.

Yu, J. M., et al. (2008). "Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo." Stem Cells Dev **17**(3): 463-473.

Mesenchymal stem cells (MSCs) have generated a great deal of interest in clinical situations, due principally to their potential use in regenerative medicine and tissue engineering applications. However, the therapeutic application of MSCs remains limited, unless the favorable effects of MSCs for tumor growth in vivo and the long-term safety of the clinical applications of MSCs can be understood more thoroughly. In this study, MSCs derived from human adipose tissues (hASCs) together with tumor cells were transplanted subcutaneously or intracranially into BALB/c nude mice to observe tumor outgrowth. The results indicated that hASCs with H460 or U87MG cells promoted tumor growth in nude mice. Our histopathological analyses indicated that the co-injection of tumor cells with hASCs exerted no influence on the formation of intratumoral vessels. Co-culture of tumor cells with hASCs or the addition of conditioned medium (CM) from hASCs effected an increase in the proliferation of H460 or U87MG cells. Co-injection of hASCs with tumor cells effected an increase in tumor cell viability in vivo, and also induced a reduction in apoptotic cell death. CM from hASCs inhibited hydrogen peroxide-induced cell death in H460 or U87MG cells. These findings indicated that MSCs could favor tumor growth in vivo. Thus, it is necessary to conduct a study concerning the long-term safety of this technique before MSCs can be used as therapeutic tools in regenerative medicine and tissue engineering.

Yuan, L., et al. (2016). "Human embryonic mesenchymal stem cells alleviate pathologic changes of MRL/Lpr mice by regulating Th7 cell differentiation." Ren Fail **38**(9): 1432-1440.

Recent evidence indicates that mesenchymal stem cells (MSC) derived from early embryonic tissues have better therapeutic ability as compared with adult tissue-derived stem cells. In the present study, we transplanted human early embryonic MSC (hMSC) into MRL/Lpr mice via tail vein injection to observe the therapeutic efficacy of hMSC and their impact on T helper 17 (Th17) cell differentiation in MRL/Lpr mice. Animals in hMSC treatment group received hMSC (1 x 10(6)/200 muL) via the tail vein at the age of 16 and 19 weeks. We found that hMSC treatment prolonged the survival of MRL/Lpr mice without inducing tumorigenesis, reduced urine protein, and alleviated the renal pathologic changes. In addition, it reduced the proportion of Th17 cells in the spleen of MRL/Lpr mice and the serum interleukin 17 (IL-17) concentration. Our in vitro experiment also demonstrated that hMSC could secrete Th17 differentiation-related cytokines of PGE2, IL-10 and TGF-beta, and IFN-gamma stimulation up-regulated the secretion of these immune regulating factors. The results of the present study suggest that hMSC therapy could alleviate systemic and local renal lesions in MRL/Lpr mice, probably by secreting immune regulating factors and regulating Th17 cell differentiation in MRL/Lpr mice.

Zemel'ko, V. I., et al. (2011). "[Multipotent mesenchymal stem cells of desquamated endometrium: isolation, characterization and use as feeder layer for maintenance of human embryonic stem cell lines]." Tsitologiia **53**(12): 919-929.

In this study, we characterize new multipotent human mesenchymal stem cell (MSC) lines derived from desquamated (shedding) endometrium in menstrual blood. The isolated endometrial MSC (eMSC) is an adhesive to plastic heterogeneous population composed mainly of endometrial glandular and stromal cells. The established cell lines meet the criteria of the International Society for Cellular Therapy for defining multipotent human MSC of any origin. The eMSCs have positive expression of CD73, CD90, CD105, CD13, CD29, CD44 markers and the absence of expression of the hematopoietic cell surface antigens CD19, CD34, CD45, CD117, CD130 and HLA-DR (class II). Multipotency of the established eMSC is confirmed by their ability to differentiate into other mesodermal cell types such as osteocytes and adipocytes. Besides, the isolated eMSC lines partially (over 50%) express the pluripotency marker SSEA-4, but do not express Oct-4. Immunofluorescent analysis

of the derived cells revealed the expression of the neural precursor markers nestin and beta-III-tubulin. This suggests a neural predisposition of the established eMSC. These cells are characterized by high rate of cell proliferation (doubling time 22-23 h) and high cloning efficiency (about 60%). In vitro the eMSCs undergo more than 45 population doublings revealing normal karyotype without karyotipic abnormalilies. We demonstrate, that the mititotically inactivated eMSCs are perfect feeder cells for human embryonic stem cell lines (hESC) C612 and C910. The eMSC being a feeder culture maintain the pluripotent status of the hESC, which is revealed by the expression of Oct-4, alkaline phosphatase and SSEA-4. When co-culturing, hESC retain their morphology, proliferative rate for more than 40 passages and capability for spontaneous differentiation into embryoid bodies comprising the three embryonic germ layers. Thus, an easy and non-invasive extraction of the eMSC in menstrual blood, their multipotency and high proliferative activity in vitro without karyotypic abnormalities demonstrate the potential of use of these stem cells in regenerative medicine. Using the derived eMSCs as the feeder culture eliminates the risks associated with animal cells while transferring hESC to clinical setting.

Zeng, Y., et al. (2013). "Electrophysiological characterisation of human umbilical cord blood-derived mesenchymal stem cells induced by olfactory ensheathing cell-conditioned medium." Neurochem Res **38**(12): 2483-2489.

Umbilical cord blood-derived marrow stromal cells (UCB-MSCs) with high proliferation capacity and immunomodulatory properties are considered to be a good candidate for cell-based therapies. But until now, little work has been focused on the differentiation of UCB-MSCs. In this work, UCB-MSCs were demonstrated to be negative for CD34 and CD45 expression but positive for CD90 and CD105 expression. The gate values of UCB-MSCs for CD90 and CD105 were 99.3 and 98.6 %, respectively. Two weeks after treatment, the percentage of neuron-like cells differentiated from UCB-MSCs was increased to 84 +/- 12 % in the experimental group [treated with olfactory ensheathing cells (OECs)-conditioned medium] and they were neuron-specific enolase positive; few neuron-like cells were found in the control group (without OECs-conditioned medium). Using whole-cell recording, sodium and potassium currents were recorded in UCB-MSCs after differentiation by OECs. Thus, human UCB-MSCs could be differentiated to neural cells by secreted secretion from OECs and exhibited electrophysiological properties similar to mature neurons after 2 weeks post-induction. These results imply that OECs can be used as a new strategy for stem cell differentiation and provide an alternative neurogenesis pathway for generating sufficient numbers of neural cells for cell therapy.

Zhang, A. Z., et al. (2017). "Cell Toxicity in Fibroblasts, Tenocytes, and Human Mesenchymal Stem Cells-A Comparison of Necrosis and Apoptosis-Inducing Ability in Ropivacaine, Bupivacaine, and Triamcinolone." Arthroscopy **33**(4): 840-848.

PURPOSE: To analyze the ability of ropivacaine, bupivacaine, and triamcinolone to induce apoptosis and necrosis in fibroblasts, tenocytes, and human mesenchymal stem cells. METHODS: Human dermal fibroblasts, adipose-derived human mesenchymal stem cells (hMSCs), and tenocytes gained from the rotator cuff tendon were seeded with a cell density of 0.5 x 10(4)/cm(2). One specimen of ropivacaine, bupivacaine, and triamcinolone was tested separately on the cells with separate concentrations of 0.5%, 0.25%, and 0.125% for each specimen. The negative control received no agent, only a change of medium. The incubation period for each agent was 30 minutes. After a change of medium and 1 hour, 24 hours, and 7 days of incubation, 10(4) cells were harvested and analyzed via fluorescence-activated cell sorting with double-staining with annexin V and propidium iodide. Statistical analysis to determine significant difference ($P < .05$) between the groups with SPSS statistics 23 through one-way analysis of variance with a univariate general linear model was performed. RESULTS: Bupivacaine showed necrosis-inducing effects on fibroblasts and tenocytes, with the necrotic effect peaking at 0.5% and 0.25%. Ropivacaine and triamcinolone caused no significant necrosis. Compared with fibroblasts and tenocytes, hMSCs did not show significant necrotic or apoptotic effects after exposure to bupivacaine. Overall, no significant differences in apoptosis were detected between different cell lines, varying concentrations, or time measurements. CONCLUSIONS: Bupivacaine 0.5% and 0.25% have the most necrosis-inducing effects on fibroblasts and tenocytes. Ropivacaine caused less necrosis than bupivaine. Compared with fibroblasts and tenocytes, hMSCs were not affected by necrosis using any of the tested agents. A significant apoptosis-inducing effect could not be detected for the different cell lines. CLINICAL RELEVANCE: Possible cell toxicity raises questions of concern for intra-articular injections using local anesthetics and corticosteroids. The present study demonstrates the necrotic and apoptotic effects of ropivacaine, bupivacaine, and triamcinolone and may give recommendations for intra-articular use of local anesthetics and corticosteroids.

Zhao, Y., et al. (2013). "Hydrogen sulfide augments the proliferation and survival of human induced pluripotent stem cell-derived mesenchymal stromal cells through inhibition of BKCa." Cytotherapy **15**(11): 1395-1405.

BACKGROUND: Hydrogen sulfide (H2S) is an endogenously generated gaseous transmitter known for its cytoprotective effect mediated by the PI3K-Akt signaling pathway. Human induced pluripotent stem cell (hiPSC)-derived mesenchymal stromal cells (MSCs), or hiPSC-MSCs, represent an alternative source of MSCs for autologous cell therapy. The big-conductance Ca(2+)-activated outward K(+) currents (BKCa), known to mediate cell proliferation, have been detected in >80% of hiPSC-MSCs. The present study aimed to explore the effect of H2S on survival and proliferation of hiPSC-MSCs and investigate the mediatory role of BKCa. METHODS: Effects of H2S on proliferation and survival of hiPSC-MSCs were measured by 5-bromo-2-deoxyuridine incorporation, population doubling and cell cycle assays, and by 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide assay and 4'-6-diamidino-2-phenylindole staining, respectively. BKCa was recorded by means of the whole-cell patch-clamp technique. The expressions of KCa 1.1 (encoding BKCa) and apoptosis-related genes were measured by reverse transcriptase-polymerase chain reaction. The phosphorylation of Akt was assessed by Western blot analysis. RESULTS: Exogenously administered NaHS (an H2S donor, 50-300 mumol/L) significantly promoted proliferation of hiPSC-MSCs. NaHS prevented the hypoxia-induced apoptosis and suppressed BKCa currents without altering the expression levels of alpha- and beta-KCa 1.1. In addition, NaHS increased the phosphorylation of Akt and decreased the expression of Caspase 8 and Bax in hiPSC-MSCs. Paxilline (1 mumol/L), a BKCa blocker, showed similar effects on promoting cell proliferation and phosphorylation of Akt and suppression of apoptotic genes in hiPSC-MSCs. CONCLUSIONS: Our data confirmed that H2S arguments the proliferation and survival of hiPSC-MSCs through activation of the PI3K-Akt pathway and that such effects could be mediated through inhibition of BKCa.

Zhou, L., et al. (2018). "SIRT1-dependent anti-senescence effects of cell-deposited matrix on human umbilical cord mesenchymal stem cells." J Tissue Eng Regen Med **12**(2): e1008-e1021.

Human umbilical cord-derived mesenchymal stem cells (UC-MSCs) are considered an attractive cell source for tissue regeneration. However, environmental oxidative stress can trigger premature senescence in MSCs and thus compromises their regenerative potential. Extracellular matrix (ECM) derived from MSCs has been shown to facilitate cell proliferation and multi-lineage differentiation. This investigation evaluated the effect of cell-deposited decellularized ECM (DECM) on oxidative stress-induced premature senescence in UC-MSCs. Sublethal dosages of H2 O2 , ranging from 50 mum to 200 mum, were used to induce senescence in MSCs. We found that DECM protected UC-MSCs from oxidative stress-induced premature senescence. When treated with H2 O2 at the same concentration, cell proliferation of DECM-cultured UC-MSCs was twofold higher than those on standard tissue culture polystyrene (TCPS). After exposure to 100 mum H2 O2 , fewer senescence-associated beta-galactosidase-positive cells were observed on DECM than those on TCPS (17.6 +/- 4.0% vs. 60.4 +/- 6.2%). UC-MSCs cultured on DECM also showed significantly lower levels of senescence-related regulators, such as p16(INK4alpha) and p21. Most importantly, DECM preserved the osteogenic differentiation potential of UC-MSCs with premature senescence. The underlying molecular mechanisms involved the silent information regulator type 1 (SIRT1)-dependent signalling pathway, confirmed by the fact that the SIRT1 inhibitor nicotinamide counteracted the DECM-mediated anti-senescent effect. Collagen type I, rather than fibronectin, partially contributed to the protective effect of decellularized matrix. These findings provide a new strategy of using stem cell-deposited matrix to overcome the challenge of cellular senescence and to facilitate the clinical application of MSCs in regenerative medicine. Copyright (c) 2017 John Wiley & Sons, Ltd.

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