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Stem Cell

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Stem Cell Research Literatures (4)

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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.

Ahrens, N., et al. (2004). "Mesenchymal stem cell content of human vertebral bone marrow." Transplantation **78**(6): 925-929.

Mesenchymal stem cells (MSCs) are capable of down-regulating alloimmune responses and promoting the engraftment of hematopoietic stem cells. MSCs may therefore be suitable for improving donor-specific tolerance induction in solid-organ transplantation. Cells from cadaveric vertebral bone marrow (V-BM), aspirated iliac crest-BM, and peripheral blood progenitor cells were compared. Cells were characterized by flow cytometry and colony assays. MSCs generated from V-BM were assayed for differentiation capacity and immunomodulatory function. A median 5.7 x 10(8) nucleated cells (NCs) were recovered per vertebral body. The mesenchymal progenitor, colony-forming unit-fibroblast, frequency in V-BM (11.6/10(5) NC, range: 6.0-20.0) was considerably higher than in iliac crest-BM (1.4/10(5) NC, range: 0.4-2.6) and peripheral blood progenitor cells (not detectable). MSC generated from V-BM had the typical MSC phenotype (CD105(pos)CD73(pos)CD45(neg)CD34(neg)), displayed multilineage differentiation potential, and suppressed alloreactivity in mixed lymphocyte reactions. V-BM may be an excellent source for MSC cotransplantation approaches.

Alimperti, S., et al. (2014). "Serum-free spheroid suspension culture maintains mesenchymal stem cell proliferation and differentiation potential." Biotechnol Prog **30**(4): 974-983.

There have been many clinical trials recently using ex vivo-expanded human mesenchymal stem cells (MSCs) to treat several disease states such as graft-versus-host disease, acute myocardial infarction, Crohn's disease, and multiple sclerosis. The use of MSCs for therapy is expected to become more prevalent as clinical progress is demonstrated. However, the conventional 2-dimensional (2D) culture of MSCs is laborious and limited in scale potential. The large dosage requirement for many of the MSC-based indications further exacerbates this manufacturing challenge. In contrast, expanding MSCs as spheroids does not require a cell attachment surface and is

amenable to large-scale suspension cell culture techniques, such as stirred-tank bioreactors. In the present study, we developed and optimized serum-free media for culturing MSC spheroids. We used Design of Experiment (DoE)-based strategies to systematically evaluate media mixtures and a panel of different components for effects on cell proliferation. The optimization yielded two prototype serum-free media that enabled MSCs to form aggregates and proliferate in both static and dynamic cultures. MSCs from spheroid cultures exhibited the expected immunophenotype (CD73, CD90, and CD105) and demonstrated similar or enhanced differentiation potential toward all three lineages (osteogenic, chondrogenic, adipogenic) as compared with serum-containing adherent MSC cultures. Our results suggest that serum-free media for MSC spheroids may pave the way for scale-up production of MSCs in clinically relevant manufacturing platforms such as stirred tank bioreactors.

Alrefaei, G. I., et al. (2015). "Does the maternal age affect the mesenchymal stem cell markers and gene expression in the human placenta? What is the evidence?" Tissue Cell **47**(4): 406-419.

BACKGROUND: Although the human placenta is considered medical wastes, it has become a main source of stem cells. Due to their easy isolation, ability to resist immune rejection and ability to differentiate into different types of adult cells, placental stem cells are considered superior to other stem cells. OBJECTIVES: This study aimed to assess the impact of the maternal age on the expression of mesenchymal stem cell (MSC) markers CD105 and CD29 in different areas of a term human placenta and to identify the differential expression of these markers in different placental areas. SUBJECTS AND METHODS: In this comparative cross sectional study, one hundred term placentas were collected after delivery from healthy mothers divided into five groups according to their age. Placentas were processed to assess both immune- and gene-expression of CD105 and CD29 surface antigen markers. Data of the different studied age groups was compared using the Statistical Package of Social Science (SPSS) software. RESULTS: CD105 and CD29 immunoexpression in decidua basalis, fetal membrane and placental villi showed significant negative correlations with the maternal age. CD105- and CD29-positive MSCs were significantly abundant in the decidua basalis and placental villi. Real-time polymerase chain reaction results were consistent with those of the immunohistochemical study. CONCLUSION: Labeling the placenta-driven MSCs with the specific area from which the cells were taken as well as the mother's age is advised and could be helpful in controlling the quality of the cell banks as well as the favorable outcome of the therapeutic applications.

Alrefaei, G. I., et al. (2015). "Effects of maternal age on the expression of mesenchymal stem cell markers in the components of human umbilical cord." Folia Histochem Cytobiol **53**(3): 259-271.

INTRODUCTION: Although the human umbilical cord (UC) has been previously considered a medical waste, its use as a main source of fetal stem cells for regenerative medicine applications has increased over the past few years. The aim of the study was to assess the impact of the maternal age on the expression of mesenchymal stem cells (MSC) markers CD105 and CD29 in the different areas of human UC. MATERIAL AND METHODS: In this comparative cross sectional study, one hundred term UCs from five maternal age groups (20-45 years) were collected after delivery from healthy mothers and were processed to assess both immuno- and gene expression of CD105 and CD29 surface antigen markers using immunohistochemical and RT-PCR techniques. RESULTS: The immunoexpression of CD105 and CD29 in the amniotic membrane (AM) and Wharton's jelly (WJ), the umbilical artery (UA) and the umbilical vein (UV) showed significant negative correlation with the maternal age ($p < 0.001$). Reduced amount of cells as well as the studied MSC markers and their gene expression levels were documented in older age mothers. CD105-positive MSCs were more abundant in the UA, whereas CD29-positive MSCs were more abundant in the AM and WJ. CONCLUSION: The decreased expression of CD105 and CD29 MSCs markers with age suggests that selective isolation of MSCs from Wharton's jelly, umbilical artery or umbilical vein of younger mothers should be recommended.

Anam, K. and T. A. Davis (2013). "Comparative analysis of gene transcripts for cell signaling receptors

in bone marrow-derived hematopoietic stem/progenitor cell and mesenchymal stromal cell populations." Stem Cell Res Ther **4**(5): 112.

INTRODUCTION: Knowing the repertoire of cell signaling receptors would provide pivotal insight into the developmental and regenerative capabilities of bone marrow cell (BMC)-derived hematopoietic stem/progenitor cells (HSPCs) and bone marrow mesenchymal stromal cells (BMMSCs). METHODS: Murine HSPCs were enriched from fluorescence-activated cell sorting (FACS)-sorted Lin-c-Kit+Sca-1+ BMCs isolated from the tibia and femoral marrow compartments. Purified BMMSCs (CD73+, CD90+, CD105+, and CD45-, CD34-, CD31-, c-Kit-) with extensive self-renewal potential and multilineage differentiation capacity (into different mesodermal cell lineages including osteocytes, chrondrocytes, adipocytes) were derived from adherent BMC cultures after CD45+ cell depletion. Adherent colony-forming cells were passaged two to three times and FACS analysis was used to assess cell purity and validate cell-specific surface marker phenotype prior to experimentation. Gene transcripts for a number of cell signaling molecules were assessed using a custom quantitative real-time RT-PCR low-density microarray (94 genes; TaqMan(R) technology). RESULTS: We identified 16 mRNA transcripts that were specifically expressed in BMC-derived HSPC (including Ptprc, c-Kit, Csf3r, Csf2rb2, Ccr4, Cxcr3 and Tie-1), and 14 transcripts specifically expressed in BMMSCs (including Pdgfra, Ddr2, Ngfr, Mst1r, Fgfr2, Epha3, and Ephb3). We also identified 27 transcripts that were specifically upregulated (>/=2-fold expression) in BMMSCs relative to HSPCs (Axl, Bmpr1a, Met, Pdgfrb, Fgfr1, Mertk, Cmkor1, Egfr, Epha7, and Ephb4), and 19 transcripts that were specifically upregulated in HSPCs relative to BMMSCs (Ccr1, Csf1r, Csf2ra, Epor, IL6ra, and IL7r). Eleven transcripts were equally expressed (<2-fold upregulation) in HSPCs and BMMSCs (Flt1, Insr, Kdr, Jak1, Agtrl1, Ccr3, Ednrb, Il3ra, Hoxb4, Tnfrsf1a, and Abcb1b), whilst another seven transcripts (Epha6, Epha8, Musk, Ntrk2, Ros1, Srms, and Tnk1) were not expressed in either cell population. CONCLUSIONS: We demonstrate that besides their unique immunophenotype and functional differences, BMC-derived HSPCs and BMMSCs have different molecular receptor signaling transcript profiles linked to cell survival, growth, cell differentiation status, growth factor/cytokine production and genes involved in cell migration/trafficking/adhesion that may be critical to maintain their pluripotency, plasticity, and stem cell function.

Aoki, S., et al. (2009). "Non-skin mesenchymal cell types support epidermal regeneration in a mesenchymal stem cell or myofibroblast phenotype-independent manner." Pathol Int **59**(6): 368-375.

Skin-derived fibroblasts, preadipocytes and adipocytes, and non-skin-derived bone marrow stromal cells support epidermal regeneration. It remains unclear, however, whether various organ-derived mesenchymal cell (MC) types other than the aforementioned counterparts affect epidermal regeneration. Using a skin reconstruction model, it is shown here that heart-, spleen-, lung-, liver- and kidney-derived MC support epidermal regeneration by keratinocytes. Immunohistochemistry showed that these MC types described here allowed keratinocytes to express cytokeratin (CK) 10, CK14 and involucrin in a normal fashion, and to retain the epidermal progenitor cell marker, p63, within the basal layer. MC types constantly expressed vimentin, but they were heterogeneous in their expression of the mesenchymal stem cell markers, stage-specific embryonic antigen-4, CD105, CD90 and CD44, and the myofibroblast marker, alpha-smooth muscle actin. The MC types expressed keratinocyte growth factor, stromal-derived factor-1 and interleukin-6, which are all critical for dermal fibroblast-keratinocyte interaction. These results indicate that vimentin-positive MC originating from the heart, spleen, lung, liver and kidney can support epidermal regeneration without the involvement of mesenchymal stem cell and myofibroblast phenotypes of MC.

Ardianto, B., et al. (2010). "The HPB-AML-I cell line possesses the properties of mesenchymal stem cells." J Exp Clin Cancer Res **29**: 163.

BACKGROUND: In spite of its establishment from the peripheral blood of a case with acute myeloid leukemia (AML)-M1, HPB-AML-I shows plastic adherence with spindle-like morphology. In addition, lipid droplets can be induced in HPB-AML-I cells by methylisobutylxanthine, hydrocortisone, and indomethacin. These findings suggest that HPB-AML-I is similar to mesenchymal stem

cells (MSCs) or mesenchymal stromal cells rather than to hematopoietic cells. METHODS: To examine this possibility, we characterized HPB-AML-I by performing cytochemical, cytogenetic, and phenotypic analyses, induction of differentiation toward mesenchymal lineage cells, and mixed lymphocyte culture analysis. RESULTS: HPB-AML-I proved to be negative for myeloperoxidase, while surface antigen analysis disclosed that it was positive for MSC-related antigens, such as CD29, CD44, CD55, CD59, and CD73, but not for CD14, CD19, CD34, CD45, CD90, CD105, CD117, and HLA-DR. Karyotypic analysis showed the presence of complicated abnormalities, but no reciprocal translocations typically detected in AML cases. Following the induction of differentiation toward adipocytes, chondrocytes, and osteocytes, HPB-AML-I cells showed, in conjunction with extracellular matrix formation, lipid accumulation, proteoglycan synthesis, and alkaline phosphatase expression. Mixed lymphocyte culture demonstrated that CD3+ T-cell proliferation was suppressed in the presence of HPB-AML-I cells. CONCLUSIONS: We conclude that HPB-AML-I cells appear to be unique neoplastic cells, which may be derived from MSCs, but are not hematopoietic progenitor cells.

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.

Astori, G., et al. (2007). ""In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells." J Transl Med **5**: 55.

BACKGROUND: The stromal vascular fraction (SVF) is a heterogeneous cell population derived from the adipose tissue. There is still a lack of information concerning the characterization of the cell subpopulations constituting the SVF as well as its mesenchymal and haematopoietic potential. Furthermore there are great variations in its phenotypical characterization. METHODS: Composition of SVF was investigated by FACS analysis, cytological and "in vitro" assays. We studied CD34+ population by combining FACS with human CFC (colony-forming-cell haematopoietic assay). The endothelial fraction was investigated by

quantifying the co-expression of specific markers (CD146, CD105, CD31 and UEA-1). Mesenchymal potential was assessed by CFU-F assay and cultured AT-MSC were characterized by a 5-color FACS analysis. The multipotent differentiation potential (osteogenic, adipogenic and chondrogenic) was investigated both at cellular and molecular level. RESULTS: We identified in the SVF two CD34+ populations with a marked difference in the intensity of antigen expression, the majority of the cells expressing CD34 at low intensity. Moreover, two CD146+ cell populations were clearly distinguishable in the SVF:a CD146 dim accounting for 9.9% of the total SVF cells and a CD146+ bright cell population accounting for about 39.3%. The frequency of CFC clones was comparable with the one reported for peripheral blood. Endothelial cells account for about 7.7% of the SVF cells. AT-MSC differenced in the osteogenic adipogenic and chondrogenic lineage. CONCLUSION: The SVF is not a homogeneous cell population, and its final composition could be influenced both by the flow cytometric technique analysis and the SVF extraction steps. The CFU-F frequency in the SVF was 1/4880, a value about seven times greater than the data reported for bone marrow. The antigenic profile of AT-MSC was comparable with bone-marrow derived MSC. AT-MSC were able to differentiate along the osteogenic adipogenic and chondrogenic lineages. The data here reported, further contribute to the characterization of SVF, a tissue providing an alternative as a source of MSC for clinical applications.

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.

Bian, Z. Y., et al. (2009). "Increased number of mesenchymal stem cell-like cells in peripheral blood of patients with bone sarcomas." Arch Med Res **40**(3): 163-168.

BACKGROUND AND AIMS: The number of peripheral blood mesenchymal stem cells (PBMSCs) may increase under pathological conditions. We sought to compare the number of MSC-like cells in the peripheral blood of patients with bone sarcomas with healthy controls and to analyze related cytokines in the peripheral blood plasma. METHODS: Peripheral blood mononuclear cells (PBMNs) of patients with bone sarcomas and control subjects were isolated for culture and analyzed by flow cytometry for MSC phenotype. Cytokines in the plasma obtained after cell separation were analyzed using enzyme-linked immunosorbent assay (ELISA). Annexin-V and beta-galactosidase staining were used to

investigate whether the cells died from apoptosis or senescence. RESULTS: Flow cytometric analysis demonstrated an >9-fold increase in the number of cells with MSC-like phenotypes (CD34(-), CD45(-), CD105(+)) in patients with bone sarcomas compared with control subjects ($p<0.05$). ELISA results showed that concentrations of hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in patients with bone sarcomas were statistically higher than those in the control subjects ($p<0.05$), whereas there was no significant difference in plasma concentrations of leptin and stromal cell-derived factor 1 between the two groups. A significant, positive correlation between the percentages of PBMSC-like cells and concentrations of HGF in all samples ($R=0.618$; $p=0.011$). Annexin-V staining of MSC-like cells was positive, whereas beta-galactosidase staining was negative. CONCLUSIONS: Peripheral blood of patients with bone sarcomas has more cells with MSC phenotypes than blood of healthy persons. The increased number is accompanied by increased HGF and VEGF in the plasma.

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.

Boyd, N. L., et al. (2009). "Human embryonic stem cell-derived mesoderm-like epithelium transitions to mesenchymal progenitor cells." Tissue Eng Part A **15**(8): 1897-1907.

Human embryonic stem cells (hESC) have the potential to produce all of the cells in the body. They are able to self-renew indefinitely, potentially making them a source for large-scale production of therapeutic cell lines. Here, we developed a monolayer differentiation culture that induces hESC (WA09 and BG01) to form epithelial sheets with mesodermal gene expression patterns (BMP4, RUNX1, and GATA4). These E-cadherin+ CD90low cells then undergo apparent epithelial-mesenchymal transition for the derivation of mesenchymal progenitor cells (hESC-derived mesenchymal cells [hES-MC]) that by flow

cytometry are negative for hematopoietic (CD34, CD45, and CD133) and endothelial (CD31 and CD146) markers, but positive for markers associated with mesenchymal stem cells (CD73, CD90, CD105, and CD166). To determine their functionality, we tested their capacity to produce the three lineages associated with mesenchymal stem cells and found they could form osteogenic and chondrogenic, but not adipogenic lineages. The derived hES-MC were able to remodel and contract collagen I lattice constructs to an equivalent degree as keloid fibroblasts and were induced to express alpha-smooth muscle actin when exposed to transforming growth factor (TGF)-beta1, but not platelet derived growth factor-B (PDGF-B). These data suggest that the derived hES-MC are multipotent cells with potential uses in tissue engineering and regenerative medicine and for providing a highly reproducible cell source for adult-like progenitor cells.

Burns, J. S., et al. (2008). "The histopathology of a human mesenchymal stem cell experimental tumor model: support for an hMSC origin for Ewing's sarcoma?" Histol Histopathol **23**(10): 1229-1240.

Sarcomas display varied degrees of karyotypic abnormality, vascularity and mesenchymal differentiation. We have reported that a strain of telomerized adult human bone marrow mesenchymal stem cells (hMSC-TERT20) spontaneously evolved a tumorigenic phenotype after long-term continuous culture. We asked to what extent our hMSC-TERT20 derived tumors reflected events found in human sarcomas using routine histopathological procedures. Early versus late passage hMSC-TERT20 cultures persistently expressed mesenchymal lineage proteins e.g. CD105, CD44, CD99 and vimentin. However, late passage cultures, showed increased immunohistochemical staining for CyclinD1 and p21WAF1/Cip1, whereas p27Kip1 staining was reduced. Notably, spectral karyotyping showed that tumorigenic hMSC-TERT20 cells retained a normal diploid karyotype, with no detectable chromosome abnormalities. Consistent with the bone-forming potential of early passage hMSC-TERT20 cells, tumors derived from late passage cells expressed early biomarkers of osteogenesis. However, hMSC-TERT20 cells were heterogeneous for alpha smooth muscle actin (ASMA) expression and one out of six hMSC-TERT20 derived single cell clones was strongly ASMA positive. Tumors from this ASMA+ clone had distinctive vascular qualities with hot spots of high CD34+ murine endothelial cell density, together with CD34- regions with a branching periodic acid Schiff reaction pattern. Such clone-specific differences in host vascular response provide novel models to explore interactions between mesenchymal stem and endothelial cells. Despite the lack of a characteristic chromosomal translocation, the histomorphology, biomarkers and oncogenic changes were similar to those prevalent for Ewing's sarcomas. The phenotype and ontogenesis of hMSC-TERT20 tumors was consistent with the hypothesis that sarcomas may arise from hMSC, providing a unique diploid model for exploring human sarcoma biology.

Carbone, A., et al. (2016). "Adipose-derived mesenchymal stromal (stem) cells differentiate to osteoblast and chondroblast lineages upon incubation with conditioned media from dental pulp stem cell-derived osteoblasts and auricle cartilage chondrocytes." J Biol Regul Homeost Agents **30**(1): 111-122.

The potential of adipose-derived mesenchymal stromal (stem) cells (ADSCs) to differentiate into either osteoblasts or chondrocytes is controversial. In this study we investigated the multicapacity potential of ADSCs to differentiate towards adipocyte, osteoblast, and chondrocyte lineages when cells are seeded onto plastic in comparison with incubation with conditioned media (CM) obtained from differentiated cell types.ADSCs, obtained from liposuctions, were characterized for mesenchymal and hematopoietic markers by cytofluorimetry. Their differentiation capacity towards adipocytes, osteoblasts, and chondrocytes was investigated by histochemistry methods (Oil-Red-O staining, Safranin O and Alizarin Red staining, respectively). Dental pulp stem cells (DPSCs) and dedifferentiated auricle derived-chondrocytes were differentiated towards osteoblastic and chondrocytic lineages respectively, and the CM obtained from these cultures was used to induce differentiation of ADSCs. ADSCs were positive for mesenchymal markers (CD29, CD105, CD73, CD44), but not for hematopoietic lineage markers (CD14, CD34, CD45) and this behavior was conserved from the isolation up to the fifth passage. While ADSCs were readily differentiated in adipocytes, they were

not towards chondrocytes and osteoblastic lineages, a behavior different from that of bone marrow-derived MSCs that differentiated into the three lineages at two weeks post-induction. Only ADSCs treated with CM from cultured chondrocytes and DPSCs, produced glycosaminoglycans and mineralized matrix. These results indicate that ADSCs need growth/morphogenic factor supplementation from the tissue environment to be appropriately differentiated to mesodermic lineages.

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.

Chen, L., et al. (2009). "[Mechanism of granulocyte colony-stimulating factor for promoting cell viability of bone marrow mesenchymal stem cells.]." Sheng Li Xue Bao **61**(2): 169-174.

The present study was aimed to investigate the mechanism of the granulocyte colony-stimulating factor (G-CSF) on the viability of the bone marrow mesenchymal stem cells (MSCs). MSCs were cultured by classical whole bone marrow adhering method, and the MSCs were analyzed for the cell surface differentiation markers CD34, CD133, CD90 and CD105 by flow cytometry (FCM). The ability of the MSCs to differentiate into osteocytes and adipocytes was tested in osteogenic and adipogenic mediums, separately. The effect of G-CSF (20 mug/mL) on the passage 3 MSCs viability was evaluated by MTT method, and the molecular mechanism of the G-CSF mediated effects was assayed through the pretreatment of the signal pathway inhibitors including 50 nmol/L wortmannin (phosphatidylinoesitol 3 kinase inhibitor), 50 mumol/L PD98059 [extracellular signal-regulated-kinase1/2 (ERK1/2) inhibitor], 30 mumol/L SB203580 (p38 mitogen-activated protein kinase inhibitor), 10 mumol/L H89 (protein kinase A inhibitor), 20 mumol/L Y27632 (Rho kinase inhibitor), 1 mumol/L rapamycin [mammalian target of rapamycin (mTOR) inhibitor], 10 mmol/L straurosporine [protein kinase C (PKC) inhibitor], 6 nmol/L G0697 (PKCalpha inhibitor) and 50 mumol/L Pseudo Z (PKCzeta inhibitor). Cultured passage 3 MSCs expressed CD90 and CD105 strongly, and showed the ability of multi-differentiation into osteocytes and adipocytes. G-CSF promoted the viability of MSCs, and the promotion was completely inhibited by PKC inhibitor straurosporine and partially inhibited by wortmannin, rapamycin, PD98059, SB203580 or G0697. However, its effect was not inhibited by H89, Y27632 and Pseudo Z. It is thus suggested that the promoting effect of G-CSF on MSCs viability was closely related to AKT-mTOR-PKC signal pathway, and PKC maybe the central role in the signal pathway.

Choi, Y. H., et al. (2011). "Mesenchymal stem cells for cardiac cell therapy." Hum Gene Ther **22**(1): 3-17.

Despite refinements of medical and surgical therapies, heart failure remains a fatal disease. Myocardial infarction is the most common cause of heart failure, and only palliative measures are available to relieve symptoms and prolong the patient's life span. Because

mammalian cardiomyocytes irreversibly exit the cell cycle at about the time of birth, the heart has traditionally been considered to lack any regenerative capacity. This paradigm, however, is currently shifting, and the cellular composition of the myocardium is being targeted by various regeneration strategies. Adult progenitor and stem cell treatment of diseased human myocardium has been carried out for more than 10 years (Menasche et al., 2001; Stamm et al., 2003), and it has become clear that, in humans, the regenerative capacity of hematopoietic stem cells and endothelial progenitor cells, despite potent proangiogenic effects, is limited (Stamm et al., 2009). More recently, mesenchymal stem cells (MSCs) and related cell types are being evaluated in preclinical models of heart disease as well as in clinical trials (see Published Clinical Trials, below). MSCs have the capacity to self-renew and to differentiate into lineages that normally originate from the embryonic mesenchyme (connective tissues, blood vessels, blood-related organs) (Caplan, 1991; Prockop, 1997; Pittenger et al., 1999). The current definition of MSCs includes plastic adherence in cell culture, specific surface antigen expression (CD105(+)/CD90(+)/CD73(+), CD34(-)/CD45(-)/CD11b(-) or CD14(-)/CD19(-) or CD79alpha(-)/HLA-DR1(-)), and multilineage in vitro differentiation potential (osteogenic, chondrogenic, and adipogenic) (Dominici et al., 2006). If those criteria are not met completely, the term "mesenchymal stromal cells" should be used for marrow-derived adherent cells, or other terms for MSC-like cells of different origin. For the purpose of this review, MSCs and related cells are discussed in general, and cell type-specific properties are indicated when appropriate. We first summarize the preclinical data on MSCs in models of heart disease, and then appraise the clinical experience with MSCs for cardiac cell therapy.

Claessen, C., et al. (2015). "Equid herpesvirus 1 (EHV1) infection of equine mesenchymal stem cells induces a pUL56-dependent downregulation of select cell surface markers." Vet Microbiol **176**(1-2): 32-39.

Equid herpesvirus 1 (EHV1) is an ubiquitous alphaherpesvirus that can cause respiratory disease, abortion and central nervous disorders. EHV1 is known to infect a variety of different cell types in vitro, but its tropism for cultured primary equine mesenchymal stem cells (MSC) has never been explored. We report that equine MSC were highly permissive for EHV1 and supported lytic replication of the virus in vitro. Interestingly, we observed that an infection of MSC with EHV1 resulted in a consistent downregulation of cell surface molecules CD29 (beta1-integrin), CD105 (endoglin), major histocompatibility complex type I (MHCI) and a variable downregulation of CD172a. In contrast, expression of CD44 and CD90 remained unchanged upon wild type infection. In addition, we found that this selective EHV1-mediated downregulation of cell surface proteins was dependent on the viral protein UL56 (pUL56). So far, pUL56-dependent downregulation during EHV1 infection of equine cells has only been described for MHCI, but our present data indicate that pUL56 may have a broader function in downregulating cell surface proteins. Taken together, our results are the first to show that equine MSC are susceptible for EHV1 and that pUL56 induces downregulation of several cell surface molecules on infected cells. These findings provide a basis for future studies to evaluate the mechanisms underlying for this selective pUL56-induced downregulation and to evaluate the potential role of MSC during EHV1 pathogenesis.

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.

De Aza, P. N., et al. (2013). "The effects of Ca2SiO4-Ca3(PO4)2 ceramics on adult human mesenchymal stem cell viability, adhesion, proliferation, differentiation and function." Mater Sci Eng C Mater Biol Appl **33**(7): 4009-4020.

Bioceramic samples with osteogenic properties, suitable for use in the regeneration of hard tissue, were synthesized. The materials consisting of alpha-tricalcium phosphate (alphaTCP) and also alphaTCP doped with either 1.5 wt.% or 3.0 wt.% of dicalcium silicate (C2S) in the system Dicalcium Silicate-Tricalcium Phosphate (C2S-TCP) were obtained by solid state reaction. All materials were composed of a single phase, alphaTCP in the case of a pure material, or solid solution of C2S in alphaTCP (alphaTCPss) for the doped alphaTCP. Viability, proliferation and in vitro osteoinductive capacity were investigated by seeding, adult mesenchymal stem cells of human origin (ahMSCs) which were CD73(+), CD90(+), CD105(+), CD34(-) and CD45(-) onto the 3 substrates for 30 days. Results show a non-cytotoxic effect after applying an indirect apoptosis test (Annexin V/7-AAD staining), so ahMSCs adhered, spread, proliferated and produced extracellular matrix (Heparan-sulfate proteoglycan (HS) and osteopontin (OP)) on all the ceramics studied. Finally, the cells lost the cluster differentiation marker expression CD73, CD90 y CD105 characteristic of ahMSCs and they showed an osteoblastic phenotype (Alkaline phosphatase activity (ALP), Osteocalcin production (OC), Collagen type I expression (Col-I), and production of mineralization nodules on the extracellular matrix). These observations were more evident in the alphaTCP ceramic doped with 1.5 wt.% C2S, indicating osteoblastic differentiation as a result of the increased concentration of solid solution of C2S in alphaTCP (alphaTCPss). Overall, these results suggest that the ceramics studied are cytocompatible and they are able to induce osteoblastic differentiation of undifferentiated ahMSCs.

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.

Divya, M. S., et al. (2012). "Umbilical cord blood-derived mesenchymal stem cells consist of a unique population of progenitors co-expressing mesenchymal stem cell and neuronal markers capable of instantaneous neuronal differentiation." Stem Cell Res Ther **3**(6): 57.

INTRODUCTION: Umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) are self-renewing multipotent progenitors with the potential to differentiate into multiple lineages of mesoderm, in addition to generating ectodermal and endodermal lineages by crossing the germline barrier. In the present study we have investigated the ability of UCB-MSCs to generate neurons, since we were able to observe varying degrees of neuronal differentiation from a few batches of UCB-MSCs with very simple neuronal induction protocols whereas other batches required extensive exposure to combination of growth factors in a stepwise protocol. Our hypothesis was therefore that the human UCB-MSCs would contain multiple types of progenitors with varying neurogenic potential and that the ratio of the progenitors with high and low neurogenic potentials varies in different batches of UCB. METHODS: In total we collected 45 UCB samples, nine of which generated MSCs that were further expanded and characterized using immunofluorescence, fluorescence-activated cell sorting and RT-PCR analysis. The neuronal differentiation potential of the UCB-MSCs was analyzed with exposure to combination of growth factors. RESULTS: We could identify two different populations of progenitors within the UCB-MSCs. One population represented progenitors with innate neurogenic potential that initially express pluripotent stem cell markers such as Oct4, Nanog, Sox2, ABCG2 and neuro-ectodermal marker nestin and are capable of expanding and differentiating into neurons with exposure to simple neuronal induction conditions. The remaining population of cells, typically expressing MSC markers, requires extensive exposure to a combination of growth factors to transdifferentiate into neurons. Interesting to note was that both of these cell populations were positive for CD29 and CD105, indicating their MSC lineage, but showed prominent difference in their neurogenic potential. CONCLUSION: Our results suggest that the expanded UCB-derived MSCs harbor a small unique population of cells that express pluripotent stem cell markers along with MSC markers and possess an inherent neurogenic potential. These pluripotent progenitors later generate cells expressing neural progenitor markers and are responsible for the instantaneous neuronal differentiation; the ratio of these pluripotent marker expressing cells in a batch determines the innate neurogenic potential.

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.

Du, L., et al. (2015). "[Culturing and characterization of human gingival mesenchymal stem cells and their chemotactic responses to stromal cell-derived factor-1]." Hua Xi Kou Qiang Yi Xue Za Zhi **33**(3): 238-243.

OBJECTIVE: To investigate the expression of chemokine stromal cell-derived factor-1 (SDF-1) receptor CXCR4 in human gingival mesenchymal stem cells (GMSCs) and the migration potential of GMSCs stimulated with SDF-1. METHODS: Human GMSCs were isolated by single-cell cloning method. Their cell surface markers were characterized by flow cytometry, and the rate of colony formation was evaluated. Differentiation assay was used to detect the differentiation potential of GMSCs. The expression of chemokine SDF-l receptor CXCR4 in GMSCs was detected by immunocytochemical staining. The chemotactic effect of SDF-1 on GMSCs was detected using a 24-multiwell Transwell cell culture chamber. The number of net migrated cells was counted in different microscope fields. RESULTS: Human GMSCs possessed high self-renewal potential and formed single-cell colonies cultured in vitro. GMSCs expressed mesenchymal stem cells-associated markers CD44, CD73, CD90, CD105, and CD166, and the expression of hemopoietic stem cell surface markers CD14, CD34, and CD45 was negative. GMSCs differentiated into osteoblasts and adipocytes under defined culture conditions. The colony forming unit-fibroblastic for GMSCs was 21.4%/+/-2.8%. Immunocytochemical staining demonstrated that GMSCs expressed chemokine SDF-1 receptor CXCR4. The number of GMSCs migrating at concentrations of 100 ng.mL-1 and 200 ng.mL-1 of SDF-l in the Transwell cell culture chamber was significantly higher than that of the negative control (189.3+/-4.4, 164.6+/-4.9 cells/field vs. 47.8+/-2.5 cells/field, P<0.01). Treatment with the CXCR4 neutralizing antibody, an antagonist for CXCR4, significantly reduced the migratory effect compared with the negative controls (29.0+/-2.4 cells/field vs. 47.8+/-2.5 cells/field, P<0.01). CONCLUSION: Human GMSCs express chemokine SDF-l receptor CXCR4. SDF-1 may participate in regulating chemotaxis of human GMSCs. Results suggest that the migration induced by SDF-1 is mediated by CXCR4.

Dvorakova, J., et al. (2008). "Isolation and characterization of mesenchymal stem cell population entrapped in bone marrow collection sets." Cell Biol Int **32**(9): 1116-1125.

Bone marrow is an important source of mesenchymal stem cells (MSCs), and a promising tool for cytotherapy. MSC utilization is limited by low cell yields obtained under standard isolation protocols. Herein, used bone marrow collection sets were evaluated as a valuable source of MSCs. Adherent cells washed from the collection sets were examined for widely accepted criteria defining MSCs. Significant numbers of cells (median 9million per set in passage 1) with colony-forming activity and high proliferative potential at low seeding densities were obtained. These cells were positive for essential MSC surface molecules (CD90, CD105, CD166, CD44, CD29) and negative for most haematopoietic and endothelial cell markers (CD45, CD34, CD11a, CD235a, HLA-DR, CD144). The cells were capable of differentiation along adipogenic, osteogenic and chondrogenic pathways. Washing out bone marrow collection sets may constitute a highly ethical source of MSCs for research purposes and may be utilized also in clinical applications.

Fang, N., et al. (2008). "[Separation of immortalized mesenchymal stem cell like stromal cells of mouse embryonic aorta-gonad-mesonephros region and their biological characteristics]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **16**(3): 584-588.

To investigate the effects of microenvironment of aorta-gonad-mesonephros (AGM) on embryonic hematopoiesis, mesenchymal stem cell like stromal cells (MSC like stromal cells) derived from dorsal aorta (DA) in AGM region were separated and identified by their growth characteristics, related molecules expression and mesenchymal lineage potentials. Stromal cells from DA region in mouse embryos (E11.5) were isolated and cultured in vitro. After transfected by pSV3neo-SV40, the clones with G418 resistance were selected, and their growth characteristics were studied. The related molecules were analyzed by flow cytometry, and each clone was induced to differentiate into adipocytes, osteocytes, and

chondrocytes. The results showed that most clones (20 clones) selected in the mouse DA region held the morphology of fibroblastoid cells. mDAF3 and mDAF18 could be grown in culture for more than 50 passages with G418 resistance, both have the potential to differentiate into adipocytes, osteocytes, and chondrocytes. At the logarithmic growth period, the cell population doubling time is about 24 hours. Surface markers, such as CD29, CD44, CD105 and Sca-1 were positively detected, while low levels of CD34, CD45, and CD31 were detected. It is concluded that immortalized mDAF3 and mDAF18 have the specific phenotype and differential potency of MSC, which suggests that MSC maybe exist in mouse embryonic DA region, where the MSC like stromal cells can be used as a cell model for further research on the modulation activity of DA microenvironment for embryonic hematopoiesis.

Fickert, S., et al. (2011). "Human mesenchymal stem cell proliferation and osteogenic differentiation during long-term ex vivo cultivation is not age dependent." J Bone Miner Metab **29**(2): 224-235.

Mesenchymal stem cells (MSCs) are of major clinical interest for the development of cell-based strategies to treat musculoskeletal diseases including critical-size bone defects caused by trauma, degenerative disorders, or infections. Elderly people mainly suffer from critical-size bone defects from the rising incidence of trauma, osteoporosis, and arthroplasties. In this study we investigated the influence of donor age on proliferation and osteogenic differentiation in long-term ex vivo cultures of primary human MSCs from patients in different age groups. Fifteen patients (8 men/7 women) comprised three age groups: (I) <50 years, (II) 50-65 years, and (III) >65 years. MSCs harvested from bone marrow derived from routine surgical procedures were isolated and cultured in standard medium over eight passages. Osteogenic differentiation was induced by dexamethasone (10 nM), ascorbic acid (300 muM), and beta-glycerophosphate (3.5 mM). Osteogenic differentiation capacity of MSCs was quantified by alkaline phosphatase (ALP) activity, fluorescence-activated cell sorting (FACS) analysis of the surface markers CD9, CD90, CD54, CD166, CD105, CD44, and CD73, and RT-PCR for Coll I and II, Cbfa 1, ALP, OC, BSP1, and GAPDH genes characterized the phenotypic changes during monolayer expansion. In vitro chondrogenic differentiation was analyzed by immunohistochemistry and RT-PCR. Progenitor cells could be expanded in the long term from all bone marrow donations. FACS single staining analysis from MSCs showed no significant difference between the age groups. The surface antigen CD166 was predominantly found in all cell cultures independently of differentiation stage. Comparison of expanded and differentiated MSCs within a single age group showed that undifferentiated MSCs had higher CD44 levels. Osteogenic stimulation of MSCs was confirmed by measuring ALP activity. The highest ALP activity was found in probands of the age group >65 years. Additionally, we observed a tendency toward male-specific ALP increase during differentiation. Osteogenic marker gene expression in MSCs was detected by RT-PCR. No significant expression differences were detected between the three donor age groups. Micromass culture of MSCs resulted histologically and immunohistologically in a chondrogenic phenotype. Elderly osteoprogenitor cell donors are a highly clinically relevant patient population. In summary, cultivation leads to a reduced osteogenic differentiation capacity regardless of age. Because donor age does not affect osteogenic differentiation potential, it should not be used as an exclusion criterion for autologous transplantation of human adult MSCs.

Forni, M. F., et al. (2016). "Murine Mesenchymal Stem Cell Commitment to Differentiation Is Regulated by Mitochondrial Dynamics." Stem Cells **34**(3): 743-755.

Mouse skin mesenchymal stem cells (msMSCs) are dermis CD105(+) CD90(+) CD73(+) CD29(+) CD34(-) mesodermal precursors which, after in vitro induction, undergo chondro, adipo, and osteogenesis. Extensive metabolic reconfiguration has been found to occur during differentiation, and the bioenergetic status of a cell is known to be dependent on the quality and abundance of the mitochondrial population, which may be regulated by fusion and fission. However, little is known regarding the impact of mitochondrial dynamics on the differentiation process. We addressed this knowledge gap by isolating MSCs from Swiss female mice, inducing these cells to differentiate into osteo, chondro, and adipocytes and measuring

changes in mass, morphology, dynamics, and bioenergetics. Mitochondrial biogenesis was increased in adipogenesis, as evaluated through confocal microscopy, citrate synthase activity, and mtDNA content. The early steps of adipo and osteogenesis involved mitochondrial elongation, as well as increased expression of mitochondrial fusion proteins Mfn1 and 2. Chondrogenesis involved a fragmented mitochondrial phenotype, increased expression of fission proteins Drp1, Fis1, and 2, and enhanced mitophagy. These events were accompanied by profound bioenergetic alterations during the commitment period. Moreover, knockdown of Mfn2 in adipo and osteogenesis and the overexpression of a dominant negative form of Drp1 during chondrogenesis resulted in a loss of differentiation ability. Overall, we find that mitochondrial morphology and its regulating processes of fission/fusion are modulated early on during commitment, leading to alterations in the bioenergetic profile that are important for differentiation. We thus propose a central role for mitochondrial dynamics in the maintenance/commitment of mesenchymal stem cells.

Fossett, E., et al. (2012). "Effect of age and gender on cell proliferation and cell surface characterization of synovial fat pad derived mesenchymal stem cells." J Orthop Res **30**(7): 1013-1018.

Cell based therapies are being investigated for biological repair of a variety of disorders. Previous work has shown that mesenchymal stem cells (MSCs) from older patients have reduced proliferation rates. As age is associated with greater musculoskeletal morbidity, e.g., osteoarthritis, an optimal MSC expansion strategy is required for older patients. In this in vitro study we investigate how age and gender affect MSC proliferation rate and cell surface characterization, as well as identify a relationship between seeding density and proliferation that could be applied to therapeutic MSC uses. Synovial fat pad derived MSCs were isolated and expanded from 14 patients undergoing total knee replacements. The cells were seeded at densities between 50 and 10,000 cells/cm(2) and cell proliferation studies, flow cytometry, and cell surface staining were performed. Females were found to have consistently higher cell proliferation and cell surface marker expression. The cell surface marker CD105 had a constant expression irrespective of age. A statistically significant inverse relationship was found between seeding densities and cell proliferation rates. This study has shown that patient characteristics do effect cell proliferation rate and cell surface characterization, but as seeding density has a significant relationship with proliferation rate, it can be altered, possibly along with other cell culturing strategies, to compensate for the effects of patient factors on MSCs. We have also shown that gender affects cell proliferation and cell surface characterization, something most previous studies may have failed to identify as they group male and female patients together.

Foster, L. J., et al. (2005). "Differential expression profiling of membrane proteins by quantitative proteomics in a human mesenchymal stem cell line undergoing osteoblast differentiation." Stem Cells **23**(9): 1367-1377.

One of the major limitations for understanding the biology of human mesenchymal stem cells (hMSCs) is the absence of prospective markers needed for distinguishing them from other cells and for monitoring lineage-specific differentiation. Mass spectrometry (MS)-based proteomics has proven extremely useful for analyzing complex protein expression patterns and, when applied quantitatively, can be used to resolve subtle differences between samples. Thus, we used MS to characterize changes in expression of membrane protein markers before and after short-term induction of osteoblast (OB) differentiation in a cell model of hMSCs established by overexpression of human telomerase reverse-transcriptase gene. We identified 463 unique proteins with extremely high confidence, including all known markers of hMSCs (e.g., SH3 [CD71], SH2 [CD105], CD166, CD44, Thy1, CD29, and HOP26 [CD63]) among 148 integral membrane or membrane-anchored proteins and 159 membrane-associated proteins. Twenty-nine integrins and cell adhesion molecules, 20 receptors, and 18 Ras-related small GTPases were also identified. Upon OB differentiation, the expression levels of 83 proteins increased by at least twofold whereas the levels of another 21 decreased by at least twofold. For example, alkaline phosphatase (ALP), versican core protein, and tenascin increased 27-, 12-, and 4-fold, respectively, and fatty acid synthase decreased sixfold. The observed increases in veriscan

and ALP were confirmed using immunocytochemistry and cytochemistry. Quantitative real-time reverse transcription-polymerase chain reaction confirmed the presence of mRNA of these membrane proteins. However, with the exception of ALP, no concordance was detected between the changes in levels of gene and protein expression during OB differentiation. In conclusion, MS-based proteomics can reveal novel markers for MSCs that can be used for their isolation and for monitoring OB differentiation.

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.

Gan, E. H., et al. (2018). "Isolation of a multipotent mesenchymal stem cell-like population from human adrenal cortex." Endocr Connect **7**(5): 617-629.

BACKGROUND: The highly plastic nature of adrenal cortex suggests the presence of adrenocortical stem cells (ACSC), but the exact in vivo identity of ACSC remains elusive. A few studies have demonstrated the differentiation of adipose or bone marrow-derived mesenchymal stem cells (MSC) into steroid-producing cells. We therefore investigated the isolation of multipotent MSC from human adrenal cortex. METHODS: Human adrenals were obtained as discarded surgical material. Single-cell suspensions from human adrenal cortex (n = 3) were cultured onto either complete growth medium (CM) or MSC growth promotion medium (MGPM) in hypoxic condition. Following ex vivo expansion, their multilineage differentiation capacity was evaluated. Phenotype markers were analysed by immunocytochemistry and flow cytometry for cell-surface antigens associated with bone marrow MSCs and adrenocortical-specific phenotype. Expression of mRNAs for pluripotency markers was assessed by q-PCR. RESULTS: The formation of colony-forming unit fibroblasts comprising adherent cells with fibroblast-like morphology were observed from the monolayer cell culture, in both CM and MGPM. Cells derived from MGPM revealed differentiation towards osteogenic and adipogenic cell lineages. These cells expressed cell-surface MSC markers (CD44, CD90, CD105 and CD166) but did not express the haematopoietic, lymphocytic or HLA-DR markers. Flow cytometry demonstrated significantly higher expression of GLI1 in cell population harvested from MGPM, which were highly proliferative. They also exhibited increased expression of the pluripotency markers. CONCLUSION: Our study demonstrates that human adrenal cortex harbours a mesenchymal stem cell-like population. Understanding the cell biology of adrenal cortex- derived MSCs will inform regenerative medicine approaches in autoimmune Addison's disease.

Ge, S., et al. (2012). "Isolation and characterization of mesenchymal stem cell-like cells from healthy and inflamed gingival tissue: potential use for clinical therapy." Regen Med **7**(6): 819-832.

AIM: Postnatal mesenchymal stem cell (MSC)-like cells have previously been isolated and ex vivo-expanded from healthy gingival tissues. The aim of this research was to isolate and characterize MSC-like cells

from inflamed gingival tissues and determine whether they retain the characteristics of MSC-like cells from healthy gingival tissues. MATERIALS & METHODS: Fifteen clonal lines of MSC-like cells from three healthy gingival tissues (GMSC-H) and fifteen from three inflamed gingival tissues (GMSC-I) were generated. Bulk-cultured cell lines from healthy and inflamed gingival tissues were also established. In vitro and in vivo characterization studies of GMSC-Is were performed relative to GMSC-Hs. RESULTS: The incidence of clonogenic colony forming units-fibroblast was comparable between healthy and inflamed gingival tissues. GMSC-H and GMSC-I clones expressed MSC-associated markers CD44, CD73, CD90, CD105 and CD166. While the population doubling capacity of GMSC-Is was reduced compared with GMSC-Hs, both populations displayed a similar capacity to undergo osteogenic, adipogenic and chondrogenic differentiation in vitro. Following subcutaneous implantation in NOD/SCID mice, both GMSC-Hs and GMSC-Is formed dense connective tissue-like structures in vivo resembling natural gingival tissue. CONCLUSION: MSC-like populations exist within inflamed gingival tissue that are functionally equivalent to MSC-like cells derived from healthy gingival tissue. Given the relative abundance of inflamed gingival tissue and ease of accessibility, MSC-like cells from inflamed gingival tissues represent a newly identified population of postnatal stem cells with immense potential in tissue engineering applications.

Ghaneialvar, H., et al. (2018). "Characterization and Classification of Mesenchymal Stem Cells in Several Species Using Surface Markers for Cell Therapy Purposes." Indian J Clin Biochem **33**(1): 46-52.

Mesenchymal stem cells are multipotent cells capable of replicating as undifferentiated cells, and have the potential of differentiating into mesenchymal tissue lineages such as osteocytes, adipocytes and chondrocytes. Such lineages can then be used in cell therapy. The aim of present study was to characterize bone marrow derived mesenchymal stem cells in four different species, including: sheep, goat, human and mouse. Human bone-marrow mesenchymal stem cells were purchased, those of sheep and goat were isolated from fetal bone marrow, and those of mouse were collected by washing bone cavity of femur and tibia with DMEM/F12. Using flow-cytometry, they were characterized by CD surface antigens. Furthermore, cells of third passage were examined for their osteogenic and adipogenic differentiation potential by oil red and alizarin red staining respectively. According to the results, CD markers studied in the four groups of mesenchymal stem cells showed a different expression. Goat and sheep expressed CD44 and CD166, and weakly expressed CD34, CD45, CD105 and CD90. Similarly, human and mouse mesenchymal cells expressed CD44, CD166, CD105 and CD90 whereas the expression of CD34 and CD45 was negative. In conclusion, although all mesenchymal stem cells display plastic adherence and tri-lineage differentiation, not all express the same panel of surface antigens described for human mesenchymal stem cells. Additional panel of CD markers are necessary to characterize regenerative potential and possible application of these stem cells in regenerative medicine and implantology.

Guan, X., et al. (2014). "Evaluation of CD24 as a marker to rapidly define the mesenchymal stem cell phenotype and its differentiation in human nucleus pulposus." Chin Med J (Engl) **127**(8): 1474-1481.

BACKGROUND: Recent studies have indicated that human nucleus pulposus contain mesenchymal stem cells (NP-MSCs). However, the immunophenotypic variation of NP-MSCs in vitro was unclear. The present study was conducted to address the immunophenotypic variation of mesenchymal stem cells in nucleus pulposus under continuous proliferation in vitro and show the difference between mesenchymal stem cells and nucleus pulposus cell. METHODS: Tissue samples were obtained from thoracolumbar burst fracture patients and degenerative disc disease patients who underwent discectomy and fusion procedures. Flow cytometric and laser scanning confocal microscope (LSCM) were used to detect the variation of mesenchymal stem cells in nucleus pulposus which were expressing CD105 and CD24 in condition with or without transforming growth factor beta1 (TGF-beta1). RESULTS: More than 90% of the analyzed primary cells of mesenchymal stem cells in nucleus pulposus fulfilled the general immunophenotyping criteria for MSCs, such as CD44, CD105 and CD29, but the marker of mature NP cells characterized as CD24 was negative. In continuous cultures, the proportion of

mesenchymal stem cells which were expressing CD44, CD105 and CD29 in nucleus pulposus gradually decreased. The mesenchymal stem cells in nucleus pulposus cells were positive for CD105 and CD29, with slight positivity for CD44. The CD24 expression gradually increased in proliferation. Biparametric flow cytometry and laser scanning confocal microscopy confirmed the presence of cells which were expressing CD105 and CD24 independently, and only a small part of cells expressed both CD105 and CD24 simultaneously. TGF-beta1 could stimulate mesenchymal stem cells in nucleus pulposus to express CD24. CONCLUSIONS: Non-degenerative and degenerative NP contains mesechymal stem cells. The variation of CD24 can be used as a marker to identify the NP-MSCs differentiation into NP-like cells.

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.

Haasters, F., et al. (2009). "Morphological and immunocytochemical characteristics indicate the yield of early progenitors and represent a quality control for human mesenchymal stem cell culturing." J Anat **214**(5): 759-767.

Human mesenchymal stem cells (hMSC) are a heterogeneous cell population, which is reflected in varying morphological and biological properties. Three subpopulations with intrinsic characteristics can be distinguished: small rapidly self-renewing cells, spindle-shaped cells and large, flattened cells. Unfortunately, it has neither been possible to morphologically define these distinct cells consistently, nor to relate them to specific surface marker features. Here, the primary hMSC subpopulations of three donors are clearly defined by maximum cell diameter and area. Furthermore, these cells were stained for the putative hMSC surface markers CD105, CD90 as well as CD73, and evaluated by three-colour flow cytometry and simultaneous multicolour immunocytochemistry. Interestingly, cell cultures with a high rate of triple-positive hMSC featured a higher content of rapidly self-renewing cells. On the other hand, a higher fraction of flattened cells correlated with a loss of one or more hMSC surface markers. The expression of CD73 showed the highest heterogeneity. Immunocytochemistry further confirmed that flattened cells mainly lack CD73 expression, whereas rapidly self-renewing cells were steadily positive for all three hMSC markers. In the literature, hMSC properties are especially conceded to rapidly self-renewing cells, whereas flattened cells have been suggested to represent early stages of lineage-specific progenitors. We reveal that among the recently suggested surface markers, CD73 is the most sensitive, as it seems to be down-regulated in the early stages of differentiation. Our morphological and immunocytochemical characterization of

hMSC subpopulations indicates the yield of early multipotent hMSC and thereby provides a quality control approach for hMSC culturing.

Han, K., et al. (2008). "Human amnion-derived mesenchymal stem cells are a potential source for uterine stem cell therapy." Cell Prolif **41**(5): 709-725.

OBJECTIVES: Human amnion is an easy-to-obtain novel source of human mesenchymal stem cells, which poses little or no ethical dilemmas. We have previously shown that human amnion-derived mesenchymal (HAM) cells exhibit certain mesenchymal stem cell-like characteristics with respect to expression of stem cell markers and differentiation potentials. MATERIALS AND METHODS: In this study, we further characterized HAM cells' potential for in vivo therapeutic application. RESULTS: Flow cytometric analyses of HAM cells show that they express several stem cell-related cell surface markers, including CD90, CD105, CD59, CD49d, CD44 and HLA-ABC, but not CD45, CD34, CD31, CD106 or HLA-DR. HAM cells at the 10th passage showed normal karyotype. More interestingly, the AbdB-like HOXA genes HOXA9, HOXA10 and HOXA11 that are expressed in the mesenchyme of the developing female reproductive tract and pregnant uteri are also expressed in HAM cells, suggesting similarities between these two mesenchymal cell types. Progesterone receptor is also highly expressed in HAM cells and expression of genes or proteins in HAM cells could be manipulated with the aid of lentivirus technology or cell-permeable peptides. To test potentials of HAM cells for in vivo application, we introduced enhanced green fluorescence protein (EGFP)-expressing HAM cells to mice by intrauterine infusion (into uteri) or by intravenous injection (into the circulation). Presence of EGFP-expressing cells within the uterine mesenchyme after intrauterine infusion or in lungs after intravenous injection was noted within 1-4 weeks. CONCLUSIONS: Collectively, these results suggest that HAM cells are a potential source of mesenchymal stem cells with therapeutic potential.

Han, S. H., et al. (2014). "Effect of ectopic OCT4 expression on canine adipose tissue-derived mesenchymal stem cell proliferation." Cell Biol Int **38**(10): 1163-1173.

Enhancing the proliferative capacity of mesenchymal stem cells (MSCs) is critical for increasing their therapeutic potential in a variety of diseases. We hypothesized that lentivirus-mediated overexpression of canine octamer-binding transcription factor 4 (OCT4) might influence the proliferation of canine adipose tissue-derived MSCs (cATMSCs). cOCT4-cATMSCs were generated by transducing cATMSCs with a cOCT4-lentiviral vector. Increased expression of cOCT4 was confirmed using RT-PCR and immunoblotting. Immunophenotypic characterization using flow cytometry indicated that the CD29, CD44, CD73, CD90, and CD105 surface markers were highly expressed by both cOCT4- and mock-transduced cATMSCs (mock-cATMSCs), whereas the CD31 and CD45 markers were absent. We performed the osteogenic differentiation assay to evaluate the effects of cOCT4 overexpression on the osteogenic differentiation potential of cATMSCs. The results showed that cOCT4-cATMSCs had a much higher potential for osteogenic differentiation than mock-cATMSCs. Next, the proliferative capacities of cOCT4- and mock-cATMSCs were evaluated using a WST-1 cell proliferation assay and trypan blue exclusion. cOCT4-cATMSCs showed a higher proliferative capacity than mock-cATMSCs. Cell cycle analysis indicated that overexpression of cOCT4 in cATMSCs induced an increase in the proportion of cells in S and G2/M phases. Consistent with this, immunoblot analysis showed that cyclin D1 expression was increased in cOCT4-cATMSCs. In conclusion, our results indicate that lentivirus-mediated overexpression of cOCT4 increased the proliferative capacity of cATMSCs. OCT4-mediated enhancement of cell proliferation may be a useful method for expanding MSC population rapidly without loss of stemness.

Hao, M., et al. (2010). "[Effect of mesenchymal stem cells on multiple myeloma cells growth and inhibition of bortezomib induced cell apoptosis]." Zhonghua Xue Ye Xue Za Zhi **31**(10): 680-683.

OBJECTIVE: To investigate the role of mesenchymal stem cells (BMSCs) in multiple myeloma (MM) bone marrow (BM) microenrivonment and their effect on myeloma cells survival and bortezomib induced apoptosis. METHODS: BMSCs were derived from BM of untreated myeloma patients (MM-BMSCs) and healthy donors (HD-BMSCs), respectively. The phenotype,

proliferation time and cytokine secretion of MM-BMSCs were detected and compared with HD-BMSCs. Then BMSCs were co-cultured with myeloma cell line NCI-H929 and bortezomib in vitro. The NCI-H929 cells proliferation and bortezomib induced cell apoptosis were investigated. RESULTS: MM-BMSCs and HD-BMSCs were isolated successfully. The phenotype of MM-BMSCs was similar to that of HD-BMSCs. Expressions of CD73, CD105, CD44 and CD29 were positive, but those of CD31, CD34, CD45 and HLA-DR (< 1%) negative. The proliferation time of MM-BMSCs was longer than that of HD-BMSCs (82 h vs 62 h, P < 0.05). Moreover, over-expressions of IL-6 and VEGF in MM-BMSCs culture supernatant were detected as compared with that in HD-BMSCs [(188.8 +/- 9.4) pg/ml vs (115.0 +/- 15.1) pg/ml and (1497.2 +/- 39.7) pg/ml vs (1329.0 +/- 21.1) pg/ml, respectively]. MM- BMSCs supported survival of the myeloma cells NCI-H929 and protected them from bortezomib induced cell apoptosis. CONCLUSIONS: MM-BMSCs is benefit for myeloma cells proliferation and against cell apoptosis induced by bortezomib. Over-expression of IL-6 and VEGF maybe play a critical role in these effects.

He, Y., et al. (2013). "Nmp4/CIZ suppresses the parathyroid hormone anabolic window by restricting mesenchymal stem cell and osteoprogenitor frequency." Stem Cells Dev **22**(3): 492-500.

Parathyroid hormone (PTH) anabolic osteoporosis therapy is intrinsically limited by unknown mechanisms. We previously showed that disabling the transcription factor Nmp4/CIZ in mice expanded this anabolic window while modestly elevating bone resorption. This enhanced bone formation requires a lag period to materialize. Wild-type (WT) and Nmp4-knockout (KO) mice exhibited equivalent PTH-induced increases in bone at 2 weeks of treatment, but by 7 weeks, the null mice showed more new bone. At 3-week treatment, serum osteocalcin, a bone formation marker, peaked in WT mice, but continued to increase in null mice. To determine if 3 weeks is the time when the addition of new bone diverges and to investigate its cellular basis, we treated 10-week-old null and WT animals with human PTH (1-34) (30 mug/kg/day) or vehicle before analyzing femoral trabecular architecture and bone marrow (BM) and peripheral blood phenotypic cell profiles. PTH-treated Nmp4-KO mice gained over 2-fold more femoral trabecular bone than WT by 3 weeks. There was no difference between genotypes in BM cellularity or profiles of several blood elements. However, the KO mice exhibited a significant elevation in CFU-F cells, CFU-F(Alk)(Phos+) cells (osteoprogenitors), and a higher percentage of CFU-F(Alk)(Phos+) cells/CFU-F cells consistent with an increase in CD45-/CD146+/CD105+/nestin+ mesenchymal stem cell frequency. Null BM exhibited a 2-fold enhancement in CD8+ T cells known to support osteoprogenitor differentiation and a 1.6-fold increase in CFU-GM colonies (osteoclast progenitors). We propose that Nmp4/CIZ limits the PTH anabolic window by restricting the number of BM stem, progenitor, and blood cells that support anabolic bone remodeling.

Hennrick, K. T., et al. (2007). "Lung cells from neonates show a mesenchymal stem cell phenotype." Am J Respir Crit Care Med **175**(11): 1158-1164.

RATIONALE: Mesenchymal stem cells have been isolated from adult bone marrow, peripheral blood, adipose tissue, trabecular bone, articular synovium, and bronchial submucosa. OBJECTIVES: We hypothesized that the lungs of premature infants undergoing mechanical ventilation contain fibroblast-like cells with features of mesenchymal stem cells. METHODS: Tracheal aspirate fluid from mechanically ventilated, premature (< 30 wk gestation) infants 7 days old or younger was obtained from routine suctioning and plated on plastic culture dishes. MEASUREMENTS AND MAIN RESULTS: A total of 11 of 20 patients studied demonstrated fibroblast-like cells, which were identified as early as 6 hours after plating. Cells were found to express the mesenchymal stem cell markers STRO-1, CD73, CD90, CD105, and CD166, as well as CCR2b, CD13, prolyl 4-hydroxylase, and alpha-smooth muscle actin. Cells were negative for the hematopoietic and endothelial cell markers CD11b, CD31, CD34, or CD45. Tracheal aspirate monocyte chemoattractant protein-1/CCL2 levels were ninefold higher in aspirates in which fibroblast-like cells were found, and cells demonstrated chemotaxis in response to monocyte chemoattractant protein. Placement of cells into appropriate media resulted in adipogenic, osteogenic, and myofibroblastic differentiation. Patients from whom mesenchymal stem cells were isolated

tended to require more days of mechanical ventilation and supplemental oxygen. CONCLUSIONS: Together, these data demonstrate that tracheal aspirate fluid from premature, mechanically ventilated infants contains fibroblasts with cell markers and differentiation potential typically found in mesenchymal stem cells.

Hermida-Gomez, T., et al. (2011). "Quantification of cells expressing mesenchymal stem cell markers in healthy and osteoarthritic synovial membranes." J Rheumatol **38**(2): 339-349.

OBJECTIVE: To quantify cells expressing mesenchymal stem cell (MSC) markers in synovial membranes from human osteoarthritic (OA) and healthy joints. METHODS: Synovial membranes from OA and healthy joints were digested with collagenase and the isolated cells were cultured. Synovial membrane-derived cells were phenotypically characterized for differentiation experiments using flow cytometry to detect the expression of mesenchymal markers (CD29, CD44, CD73, CD90, CD105, CD117, CD166, and STRO-1) and hematopoietic markers (CD34 and CD45). Chondrogenesis was assessed by staining for proteoglycans and collagen type II, adipogenesis by using a stain for lipids, and osteogenesis by detecting calcium deposits. Coexpression of CD44, CD73, CD90, and CD105 was determined using immunofluorescence. RESULTS: Cells expressing MSC markers were diffusely distributed in OA synovial membranes; in healthy synovial membrane these cells were localized in the subintimal zone. More numerous MSC markers in OA synovial membranes were observed in cells also expressing the CD90 antigen. FACS analysis showed that more than 90% of OA synovial membrane-derived cells were positive for CD44, CD73, and CD90, and negative for CD34 and CD45. OA synovial membrane-derived cells were also positive for CD29 (85.23%), CD117 (72.35%), CD105 (45.5%), and STRO-1 (49.46%). Micropellet analyses showed that the culture of cells with transforming growth factor-ss3 stimulated proteoglycan and collagen type II synthesis. CONCLUSION: Synovial membranes from patients with OA contain more cells positive for CD44, CD90, and CD105 antigens than those from joints with undamaged cartilage.

Huang, A. H., et al. (2010). "[Culture and characterization of and lentiviral vectors mediated glial cell derived neurotrophic factor expression in mesenchymal stem cells from human umbilical cord blood]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao **32**(1): 39-45.

OBJECTIVE: To isolate and culture mesenchymal stem cells from umbilical cord blood (UCB-MSCs), study its biological characterization in vitro, transfect UCB-MSCs using lentiviral vectors encoding glial cell derived neurotrophic factor (GDNF) gene, evaluate the biological function change of UCB-MSCs, and detect GDNF expression level in vitro. METHODS: We isolated monocyte by Ficoll density gradient, separated two kinds of adherent cells through different trypsin digestion time, and detected the cells surface markers by fluorescence activated cell sorting when it was proliferated for P7 passages. At the same time, we sub-cloned GDNF gene into lentiviral vectors and packaged lentiviral supernatant through three plasmids co-transfection method, then transfected the UCB-MSCs using lentiviral vectors encoding GDNF at different multiplicity of infection, and evaluated the change of biological function by observing the ability of proliferation and differentiation, morphology, and the cells surface markers. We detected the GDNF mRNA and protein expression level by using real-time polymerase chain reaction (real-time PCR) and enzyme-link immunosorbent assay (ELISA). RESULTS: The UCB-MSCs were successfully isolated and cultured in vitro, and induced it to differentiate into fat cells. FACS results showed that the UCB-MSCs expressed CD90, CD73, and CD105 positively, and CD14, CD34, CD45, CD19, HLA-DR, Stro-1, and CD106 negatively. Real-time PCR and ELISA showed that the expressions of GDNF protein and mRNA were correlated with the copy number of transfected cells: high copy number of transfected cells were associated with high GDNF expression. The biological characterization of UCB-MSCs did not obviously change after sub-cloning with GDNF. CONCLUSIONS: UCB-MSCs was successfully isolated and cultured in vitro. By transfecting UCB-MSCs with GDNF gene-containing lentiviral vectors, the secretion of GDNF protein and mRNA expression level can be controlled by the copy number of transfected cells, and thus make it constantly express GDNF at high level.

Hussain, I., et al. (2012). "New approach to isolate mesenchymal stem cell (MSC) from human umbilical cord blood." Cell Biol Int **36**(7): 595-600.

HUCB (human umbilical cord blood) has been frequently used in clinical allogeneic HSC (haemopoietic stem cell) transplant. However, HUCB is poorly recognized as a rich source of MSC (mesenchymal stem cell). The aim of this study has been to establish a new method for isolating large number of MSC from HUCB to recognize it as a good source of MSC. HUCB samples were collected from women following their elective caesarean section. The new method (Clot Spot method) was carried out by explanting HUCB samples in mesencult complete medium and maintained in 37 degrees C, in a 5% CO2 and air incubator. MSC presence was established by quantitative and qualitative immunophenotyping of cells and using FITC attached to MSC phenotypic markers (CD29, CD73, CD44 and CD105). Haematopoietic antibodies (CD34 and CD45) were used as negative control. MSC differentiation was examined in neurogenic and adipogenic media. Immunocytochemistry was carried out for the embryonic markers: SOX2 (sex determining region Y-box 2), OLIG-4 (oligodendrocyte-4) and FABP-4 (fatty acid binding protein-4). The new method was compared with the conventional Rosset Sep method. MSC cultures using the Clot Spot method showed 3-fold increase in proliferation rate compared with conventional method. Also, the cells showed high expression of MSC markers CD29, CD73, CD44 and CD105, but lacked the expression of specific HSC markers (CD34 and CD45). The isolated MSC showed some differentiation by expressing the neurogenic (SOX2 and Olig4) and adipogenic (FABP-4) markers respectively. In conclusion, HUCB is a good source of MSC using this new technique.

Hynes, K., et al. (2014). "Generation of functional mesenchymal stem cells from different induced pluripotent stem cell lines." Stem Cells Dev **23**(10): 1084-1096.

The therapeutic potential of mesenchymal stem cells (MSC) has highlighted the need for identifying easily accessible and reliable sources of these cells. An alternative source for obtaining large populations of MSC is through the controlled differentiation of induced pluripotent stem cells (iPSC). In the present study, colonies of iPSC were cultured in MSC culture media for 2 weeks. Serial passaging then selected for fast growing MSC-like cells with a typical fibroblastic morphology and the capacity to proliferate on standard culture flasks without feeder cells. MSC-like cells were developed from iPSC lines arising from three different somatic tissues: gingiva, periodontal ligament (PDL), and lung. The iPSC-MSC like cells expressed key MSC-associated markers (CD73, CD90, CD105, CD146, and CD166) and lacked expression of pluripotent markers (TRA160, TRA181, and alkaline phosphatase) and hematopoietic markers (CD14, CD34, and CD45). In vitro iPSC-MSC-like cells displayed the capacity to differentiate into osteoblasts, adipocytes, and chondrocytes. In vivo subcutaneous implantation of the iPSC-MSC-like cells into NOD/SCID mice demonstrated that only the PDL-derived iPSC-MSC-like cells exhibited the capacity to form mature mineralized structures which were histologically similar to mature bone. These findings demonstrate that controlled induction of iPSC into fibroblastic-like cells that phenotypically and functionally resemble adult MSC is an attractive approach to obtain a readily available source of progenitor cells for orthopedic and dental-related tissue-engineering applications. However, a detailed characterization of the iPSC-MSC-like cells will be important, as MSC-like cells derived from different iPSC lines exhibit variability in their differentiation capacity.

Ishimura, D., et al. (2008). "Differentiation of adipose-derived stromal vascular fraction culture cells into chondrocytes using the method of cell sorting with a mesenchymal stem cell marker." Tohoku J Exp Med **216**(2): 149-156.

The incidence of arthritic diseases is rapidly increasing in most advanced countries. Articular cartilage, which is the most important tissue in the joint, consists of chondrocytes and abundant extracellular matrix, including aggrecan, and shows poor self-repair. We studied the potential of stem cells in mouse subcutaneous adipose tissue as a source of cells to regenerate cartilage tissue. Analysis of adipose-derived stromal vascular fraction culture cells (ADSVFs) using mesenchymal stem cell markers showed that CD90-positive cells accounted for 93.8%, CD105-positive cells for 68.5%, and p75 neurotrophin receptor (p75NTR, CD271)-

positive cells for 36.1%. These results indicate that cells positive for mesenchymal stem cell markers are present in ADSVFs. The CD105-positive or -negative cells were isolated from ADSVFs by magnetic cell separation (MACS), and the efficiency of differentiation into chondrocytes was compared with using three methods of pellet method, gel-coating method, and gel-embedding sheet method. Using the CD105-positive cells and the gel-embedding sheet method, aggrecan mRNA was detected about three times higher than pellet and gel-coating methods. The above data suggest that ADSVFs could be differentiated into chondrocyte-like cells in the gel-embedding sheet method and could be useful in regenerative medicine to treat cartilage defects or cartilage degenerative disease. The use of cells sorted by mesenchymal stem cell markers from adipose tissue would gain position in the repair of cartilage tissue.

Ito, A., et al. (2005). "Magnetic force-based mesenchymal stem cell expansion using antibody-conjugated magnetoliposomes." J Biomed Mater Res B Appl Biomater **75**(2): 320-327.

Recently, there has been an accumulation of evidence indicating that human mesenchymal stem cells (MSCs, multipotent cells resident in the bone marrow) are useful for autologous cell transplantation. However, only small numbers of MSCs have been obtained in bone marrow aspirates. We have developed a novel methodology for enriching and proliferating MSCs from bone marrow aspirates using antibody-conjugated magnetoliposomes (AMLs). The AMLs are liposomes conjugated to anti-CD105 antibody (immunoliposomes) and contain magnetite nanoparticles (diameter 10 nm). In the present study, the AMLs were added to a small volume (1 mL) of human bone marrow aspirate. After a 1-h incubation period, the bone marrow aspirates containing AMLs were seeded into 10-cm tissue culture dishes, and a disk-shaped magnet (diameter 2.2 cm; height 1 cm; 4000 Gauss) was positioned under the dish to enrich MSCs by magnetic force. The MSCs proliferated, forming colonies at the site where the magnet was positioned. In contrast, no colonies and very few viable cells were observed in ordinary culture based on plastic-adherent tendencies of cells without use of AMLs. These results suggest that this AML culture method can rapidly and efficiently expand a small number of MSCs into numbers suitable for clinical application.

Jakubikova, J., et al. (2016). "A novel 3D mesenchymal stem cell model of the multiple myeloma bone marrow niche: biologic and clinical applications." Oncotarget **7**(47): 77326-77341.

Specific niches within the tumor bone marrow (BM) microenvironment afford a sanctuary for multiple myeloma (MM) clones due to stromal cell-tumor cell interactions, which confer survival advantage and drug resistance. Defining the sequelae of tumor cell interactions within the MM niches on an individualized basis may provide the rationale for personalized therapies. To mimic the MM niche, we here describe a new 3D co-culture ex-vivo model in which primary MM patient BM cells are co-cultured with mesenchymal stem cells (MSC) in a hydrogel 3D system. In the 3D model, MSC with conserved phenotype (CD73+CD90+CD105+) formed compact clusters with active fibrous connections, and retained lineage differentiation capacity. Extracellular matrix molecules, integrins, and niche related molecules including N-cadherin and CXCL12 are expressed in 3D MSC model. Furthermore, activation of osteogenesis (MMP13, SPP1, ADAMTS4, and MGP genes) and osteoblastogenic differentiation was confirmed in 3D MSC model. Co-culture of patient-derived BM mononuclear cells with either autologous or allogeneic MSC in 3D model increased proliferation of MM cells, CXCR4 expression, and SP cells. We carried out immune profiling to show that distribution of immune cell subsets was similar in 3D and 2D MSC model systems. Importantly, resistance to novel agents (IMiDs, bortezomib, carfilzomib) and conventional agents (doxorubicin, dexamethasone, melphalan) was observed in 3D MSC system, reflective of clinical resistance. This 3D MSC model may therefore allow for studies of MM pathogenesis and drug resistance within the BM niche. Importantly, ongoing prospective trials are evaluating its utility to inform personalized targeted and immune therapy in MM.

Jaramillo-Ferrada, P. A., et al. (2012). "Differential mesengenic potential and expression of stem cell-fate modulators in mesenchymal stromal cells from human-term placenta and bone marrow." J Cell Physiol **227**(9): 3234-3242.

Placenta has attracted increasing attention over the past decade as a stem cell source for regenerative medicine. In particular, the amniochorionic membrane has been shown to harbor populations of mesenchymal stromal cells (MSCs). In this study, we have characterized ex vivo expanded MSCs from the human amniotic (hAMSCs) and chorionic (hCMSCs) membranes of human full-term placentas and adult bone marrow (hBMSCs). Our results show that hAMSCs, hCMSCs, and hBMSCs express typical mesenchymal (CD73, CD90, CD105, CD44, CD146, CD166) and pluripotent (Oct-4, Sox2, Nanog, Lin28, and Klf4) markers but not hematopoietic markers (CD45, CD34). Ex vivo expanded hAMSCs were found to be of fetal origin, while hCMSCs cultures contained only maternal cells. Cell proliferation was significantly higher in hCMSCs, compared to hAMSCs and hBMSCs. Integrin profiling revealed marked differences in the expression of alpha subunits between the three cell sources. Cadherin receptors were consistently expressed on a subset of progenitors (ranging from 1% to 60%), while N-CAM (CD56) was only expressed in hAMSCs and hCMSCs but not in hBMSCs. When induced to differentiate, hAMSCs and hCMSCs displayed strong chondrogenic and osteogenic differentiation potential but very limited capacity for adipogenic conversion. In contrast, hBMSCs showed strong differentiation potential along the three lineages. These results illustrate how MSCs from different ontological sources display differential expression of cell-fate mediators and mesodermal differentiation capacity.

Jeon, M. S., et al. (2011). "Characterization of mouse clonal mesenchymal stem cell lines established by subfractionation culturing method." World J Stem Cells **3**(8): 70-82.

AIM: To characterize single-cell-derived mouse clonal mesenchymal stem cells (mcMSCs) established with bone marrow samples from three different mouse strains. METHODS: We established mcMSC lines using subfractionation culturing method from bone marrow samples obtained from long bones. These lines were characterized by measuring cell growth, cell surface epitopes, differentiation potential, lineage-specific gene expression and T-cell suppression capability. Nonclonal MSCs isolated by the conventional gradient centrifugation method were used as controls. RESULTS: All mcMSC lines showed typical nonclonal MSC-like spindle shape morphology. Lines differed in optimal growth density requirement. Cell surface epitope profiles of these mcMSC lines were similar to those of nonclonal MSCs. However, some lines exhibited different expression levels in a few epitopes, such as CD44 and CD105. Differentiation assays showed that 90% of the mcMSC lines were capable of differentiating into adipogenic and/or chondrogenic lineages, but only 20% showed osteogenic lineage differentiation. T-cell suppression analysis showed that 75% of the lines exhibited T-cell suppression capability. CONCLUSION: mcMSC lines have similar cell morphology and cell growth rate but exhibit variations in their cell surface epitopes, differentiation potential, lineage-specific gene expression and T-cell suppression capability.

Kang, J., et al. (2014). "Control of mesenchymal stem cell phenotype and differentiation depending on cell adhesion mechanism." Eur Cell Mater **28**: 387-403.

Control of cell-matrix adhesion has become an important issue in the regulation of stem cell function. In this study, a maltose-binding protein (MBP)-linked basic fibroblast growth factor (FGF2)-immobilised polystyrene surface (PS-MBP-FGF2) was applied as an artificial matrix to regulate integrin-mediated signalling. We sought to characterise human mesenchymal-stem cell (hMSC) behaviour in response to two different mechanisms of cell adhesion; (i) FGF2-heparan sulphate proteoglycan (HSPG)-mediated adhesion vs. (ii) fibronectin (FN)-integrin-mediated adhesion. Heparin inhibited hMSC adhesion to PS-MBP-FGF2 but not to FN-coated surface. The phosphorylation of focal adhesion kinase, cytoskeletal re-organisation, and cell proliferation were restricted in hMSCs adhering to PS-MBP-FGF2 compared to FN-coated surface. Expression of MSC markers, such as CD105, CD90 and CD166, decreased in hMSCs expanded on PS-MBP-FGF2 compared to expression in cells expanded on FN-coated surface. hMSCs that were expanded on FN-coated surface differentiated into osteogenic and adipogenic cells more readily than those that were expanded on PS-MBP-FGF2. Furthermore, we characterised the N-linked glycan structures of hMSCs depending on the cell adhesion mechanism using mass spectrometry (MS)-based quantitative techniques. MS analysis

revealed that 2,3-sialylated glycans, a potential marker of stem cell function, were more abundant on hMSCs expanded on FN-coated surface than on those expanded on PS-MBP-FGF2. Thus, the differentiation potential of hMSCs is controlled by the type of adhesion substrate that might provide an idea for the design of biomaterials to control stem cell fate. Elucidation of the glycan structure on the cell membrane may help characterise hMSC function.

Kara, M., et al. (2015). "A Role for MOSPD1 in Mesenchymal Stem Cell Proliferation and Differentiation." Stem Cells **33**(10): 3077-3086.

Mesenchymal stem cells (MSCs) isolated from many tissues including bone marrow and fat can be expanded in vitro and can differentiate into a range of different cell types such as bone, cartilage, and adipocytes. MSCs can also exhibit immunoregulatory properties when transplanted but, although a number of clinical trials using MSCs are in progress, the molecular mechanisms that control their production, proliferation, and differentiation are poorly understood. We identify MOSPD1 as a new player in this process. We generated MOSPD1-null embryonic stem cells (ESCs) and demonstrate that they are deficient in their ability to differentiate into a number of cell lineages including osteoblasts, adipocytes, and hematopoietic progenitors. The self-renewal capacity of MOSPD1-null ESCs was normal and they exhibited no obvious defects in early germ layer specification nor in epithelial to mesenchymal transition (EMT), indicating that MOSPD1 functions after these key steps in the differentiation process. Mesenchymal stem cell (MSC)-like cells expressing CD73, CD90, and CD105 were generated from MOSPD1-null ESCs but their growth rate was significantly impaired implying that MOSPD1 plays a role in MSC proliferation. Phenotypic deficiencies exhibited by MOSPD1-null ESCs were rescued by exogenous expression of MOSPD1, but not MOSPD3 indicating distinct functional properties of these closely related genes. Our in vitro studies were supported by RNA-sequencing data that confirmed expression of Mospd1 mRNA in cultured, proliferating perivascular pre-MSCs isolated from human tissue. This study adds to the growing body of knowledge about the function of this largely uncharacterized protein family and introduces a new player in the control of MSC proliferation and differentiation.

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.

Katebi, M., et al. (2009). "Adenosine A2A receptors play an active role in mouse bone marrow-derived mesenchymal stem cell development." J Leukoc Biol **85**(3): 438-444.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) play a role in wound healing and tissue repair and may also be useful for organ regeneration. As we have demonstrated previously that A(2A) adenosine receptors (A(2A)R) promote tissue repair and wound healing by stimulating local repair mechanisms and enhancing accumulation of endothelial progenitor cells, we investigated whether A(2A)R activation modulates BM-MSC proliferation and differentiation. BM-MSCs were isolated and cultured from A(2A)-deficient and ecto-5'nucleotidase (CD73)-deficient female mice; the MSCs were identified and quantified by a CFU-fibroblast (CFU-F) assay. Procollagen alpha2 type I expression was determined by Western blotting and immunocytochemistry. MSC-specific markers were examined in primary cells and third-passage cells by cytofluorography. PCR and real time-PCR were used to quantitate adenosine receptor and CD73 expression. There were significantly fewer CFU-Fs in cultures of BM-MSCs from A(2A)R knockout (KO) mice or BM-MSCs treated with the A(2A)R antagonist ZM241385, 1 microM. Similarly, there were significantly fewer procollagen alpha2 type I-positive MSCs in cultures from A(2A)R KO and antagonist-treated cultures as well. In late passage cells, there were significantly fewer MSCs from A(2A) KO mice expressing CD90, CD105, and procollagen type I ($P<0.05$ for all; n=3). These findings indicate that adenosine and adenosine A(2A)R play a critical role in promoting the proliferation and differentiation of mouse BM-MSCs.

Kawamura, M., et al. (2015). "Xenotransplantation of Bone Marrow-Derived Human Mesenchymal Stem Cell Sheets Attenuates Left Ventricular Remodeling in a Porcine Ischemic Cardiomyopathy Model." Tissue Eng Part A **21**(15-16): 2272-2280.

INTRODUCTION: Bone marrow-derived autologous human mesenchymal stem cells (MSCs) are one of the most promising cell sources for cell therapy to treat heart failure. The cell sheet technique has allowed transplantation of a large number of cells and enhanced the efficacy of cell therapy. We hypothesized that the transplantation of MSC sheets may be a feasible, safe, and effective treatment for ischemic cardiomyopathy (ICM). METHODS AND RESULTS: Human MSCs acquired from bone marrow were positive for CD73, CD90, and CD105 and negative for CD11b and CD45 by flow cytometry. Ten MSC sheets were created from a total cell number of 1x10(8) MSCs using temperature-responsive culture dishes. These were successfully transplanted over the infarct myocardium of porcine ICM models induced by placing an ameroid constrictor on the left anterior descending coronary artery without any procedural-related complications (MSC group=6: sheet transplantation; sham group=6, oral intake of tacrolimus in both groups). Premature ventricular contractions were rarely detected by Holter electrocardiogram (ECG) in the MSC group in the first week after transplantation. On echocardiography, the cardiac performance of the MSC group was significantly better than that of the sham group at 8 weeks after transplantation. On histological examination 8 weeks after transplantation, left ventricular (LV) remodeling was significantly attenuated compared with the sham group (cardiomyocyte size and interstitial fibrosis were measured). Immunohistochemistry of the von Willebrand factor showed that the vascular density in the infarct border area was significantly greater in the MSC group than the sham group. Expression of angiogenesis-related factors in the infarct border area of the MSC group was significantly greater than that of the sham group, as measured by real-time polymerase chain reaction. CONCLUSIONS: Bone marrow-derived MSC sheets improved cardiac function and attenuated LV remodeling in ICM without major complications, indicating that this strategy would be applicable in clinical settings.

Khan, W. S., et al. (2012). "Fat pad-derived mesenchymal stem cells as a potential source for cell-based adipose tissue repair strategies." Cell Prolif **45**(2): 111-120.

BACKGROUND: Mesenchymal stem cells are able to undergo adipogenic differentiation and present a possible alternative cell source for regeneration and replacement of adipose tissue. The human infrapatellar fat pad is a promising source of mesenchymal stem cells with many source advantages over from bone marrow. It is important to determine whether a potential mesenchymal stem-cell exhibits tri-lineage differentiation potential and is able to maintain its proliferation potential and cell-surface characterization on expansion in tissue culture. We have previously shown that mesenchymal stem cells derived from the fat

pad can undergo chondrogenic and osteogenic differentiation, and we characterized these cells at early passage. In the study described here, proliferation potential and characterization of fat pad-derived mesenchymal stem cells were assessed at higher passages, and cells were allowed to undergo adipogenic differentiation. MATERIALS AND METHODS: Infrapatellar fat pad tissue was obtained from six patients undergoing total knee replacement. Cells isolated were expanded to passage 18 and proliferation rates were measured. Passage 10 and 18 cells were characterized for cell-surface epitopes using a range of markers. Passage 2 cells were allowed to undergo differentiation in adipogenic medium. RESULTS: The cells maintained their population doubling rates up to passage 18. Cells at passage 10 and passage 18 had cell-surface epitope expression similar to other mesenchymal stem cells previously described. By staining it was revealed that they highly expressed CD13, CD29, CD44, CD90 and CD105, and did not express CD34 or CD56, they were also negative for LNGFR and STRO1. 3G5 positive cells were noted in cells from both passages. These fat pad-derived cells had adipogenic differentiation when assessed using gene expression for peroxisome proliferator-activated receptor gamma2 and lipoprotein lipase, and oil red O staining. DISCUSSION: These results indicate that the cells maintained their proliferation rate, and continued expressing mesenchymal stem-cell markers and pericyte marker 3G5 at late passages. These results also show that the cells were capable of adipogenic differentiation and thus could be a promising source for regeneration and replacement of adipose tissue in reconstructive surgery.

Khan, W. S. and T. E. Hardingham (2012). "The characterisation of mesenchymal stem cells: a stem cell is not a stem cell is not a stem cell." J Stem Cells **7**(2): 87-95.

There has been an increasing interest in stem cell applications and tissue engineering approaches in surgical practice to deal with damaged or lost tissue. Although there have been developments in almost all surgical disciplines, the greatest advances are being made in orthopaedics. This is due to many factors including the familiarity with bone marrow derived mesenchymal stem cells. Unfortunately significant hurdles remain to be overcome in many areas before tissue engineering becomes more routinely used in clinical practice. Stem cells have been identified in a number of adult tissues, albeit in small numbers. In addition to bone marrow, mesenchymal stem cells have been identified in a number of tissues including adipose tissue and fat pad. The mesenchymal stem cells are generally isolated from the tissue and expanded in culture. These cells are characterised or defined using a set of cell surface markers; mesenchymal stem cells are generally positive for CD44, CD90 and CD105, and are negative for haematopoetic markers CD34 and CD45, and the neurogenic marker CD56. In this paper the characterisation of stem cells is discussed followed by preliminary evidence suggesting that pericytes may be a candidate stem cell.

Kim, S. K., et al. (2016). "Combination of three angiogenic growth factors has synergistic effects on sprouting of endothelial cell/mesenchymal stem cell-based spheroids in a 3D matrix." J Biomed Mater Res B Appl Biomater **104**(8): 1535-1543.

Combinations of angiogenic growth factors have been shown to have synergistic effects on angiogenesis and natural wound healing in various animal models. Each growth factor has unique roles during angiogenesis; vascular endothelial growth factor (VEGF) plays a key role during the initial step of angiogenesis, whereas PDGF functions in the maturation of blood vessels. We used a combination of three angiogenic growth factors to increase angiogenesis in vitro and in vivo. We chose VEGF as a basic factor and added platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) to induce angiogenesis in three in vitro and in vivo models: 3D angiogenesis assay, 3D co-culture, and matrigel plug implantation assay. Cell proliferation was significantly higher in co-cultured cells treated with PDGF + VEGF + FGF than in the control, single, or dual combination groups. mRNA expression of alpha-smooth muscle actin (alpha-SMA), von Willebrand factor (vWF), and CD105 was higher in the triple group (PDGF + VEGF + FGF) than in control, single, or dual combination groups. In the PDGF + VEGF + FGF group, the length and number of branches of spheroids was also significantly higher than in the control, single, or dual combination groups. Furthermore, in a nude

mouse model, alpha-SMA expression was significantly higher in the PDGF + VEGF + FGF group than in other groups. In conclusion, the addition of PDGF and FGF to VEGF showed synergistic effects on angiogenesis in vitro and in vivo. (c) 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater, 104B: 1535-1543, 2016.

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.

Kouroupis, D., et al. (2013). "Assessment of umbilical cord tissue as a source of mesenchymal stem cell/endothelial cell mixtures for bone regeneration." Regen Med **8**(5): 569-581.

AIM: To enumerate and characterize mesenchymal stem cells (MSCs) and endothelial cells (ECs) in umbilical cord (UC) tissue digests. MATERIALS & METHODS: Cultured UC cells were characterized phenotypically, and functionally by using 48-gene arrays. Native MSCs and ECs were enumerated using flow cytometry. RESULTS: Compared with bone marrow (BM) MSCs, UC MSCs displayed significantly lower (range 4-240-fold) basal levels of bone-related transcripts, but their phenotypes were similar (CD73(+), CD105(+), CD90(+), CD45(-) and CD31(-)). UC MSCs responded well to osteogenic induction, but day 21 postinduction levels remained below those achieved by BM MSCs. The total yield of native UC MSCs (CD90(+), CD45(-) and CD235alpha(-)) and ECs (CD31(+), CD45(-) and CD235alpha(-)) exceeded 150 and 15 million cells/donation, respectively. Both UC MSCs and ECs expressed CD146. CONCLUSION: While BM MSCs are more predisposed to osteogenesis, UC tissue harbors large numbers of MSCs and ECs; such minimally manipulated 'off-the-shelf' cellular mixtures can be used for regenerating bone in patients with compromised vascular supply.

Kouroupis, D., et al. (2014). "The assessment of CD146-based cell sorting and telomere length analysis for establishing the identity of mesenchymal stem cells in human umbilical cord." F1000Res **3**: 126.

Adult stem cells are characterised by longer telomeres compared to mature cells from the same tissue. In this study, candidate CD146 (+) umbilical cord (UC) mesenchymal stem cells (MSCs) were purified by cell sorting from UC tissue digests and their telomere lengths were measured in comparison to donor-matched CD146-negative fraction. UC tissue fragments were enzymatically treated with collagenase and the cells were used for cell sorting, colony-forming fibroblast (CFU-F) assay or for long-term MSC cultivation. Telomere lengths were measured by qPCR in both culture-expanded MSCs and candidate native

UC MSCs. Immunohistochemistry was undertaken to study the topography of CD146 (+) cells. Culture-expanded UC MSCs had a stable expression of CD73, CD90 and CD105, whereas CD146 declined in later passages which correlated with the shortening of telomeres in the same cultures. In five out of seven donors, telomeres in candidate native UC MSCs (CD45 (-)CD235alpha (-)CD31 (-)CD146 (+)) were longer compared to donor-matched CD146 (-) population (CD45 (-)CD235alpha (-)CD31 (-)CD146 (-)). The frequency of CD45 (-)CD235alpha (-)CD31 (-)CD146 (+) cells measured by flow cytometry was ~1000-fold above that of CFU-Fs (means 10.4% and 0.01%, respectively). CD146 (+) cells were also abundant in situ having a broad topography including high levels of positivity in muscle areas in addition to vessels. Although qPCR-based telomere length analysis in sorted populations could be limited in its sensitivity, very high frequency of CD146 (+) cells in UC tissue suggests that CD146 expression alone is unlikely to be sufficient to identify and purify native MSCs from the UC tissue.

Kozdon, K., et al. (2015). "Mesenchymal Stem Cell-Like Properties of Orbital Fibroblasts in Graves' Orbitopathy." Invest Ophthalmol Vis Sci **56**(10): 5743-5750.

PURPOSE: Graves' orbitopathy (GO) is a sight-threatening autoimmune disorder causing extraocular muscle fibrosis, upper lid retraction and eye bulging due to orbital fat expansion. These clinical features are mediated by aspects of orbital fibroblasts differentiation, including adipogenesis and fibrosis. Our previous work suggested that this dual phenotype might be a manifestation of mixed cell populations, partially linked to the expression of mesenchymal stem cell (MSC) marker CD90. Thus, we set out to determine whether GO orbital fibroblasts displayed MSC properties. METHODS: Control and GO orbital fibroblasts previously characterized for CD90 and CD45 expression were analyzed by flow cytometry for classical MSC positive (CD73, CD105) and negative (CD14, CD19, HLA-DR, and CD34) markers. Graves' orbitopathy fibroblasts were tested further for their ability to undergo lineage specific differentiation following standard protocols. RESULTS: Control and GO fibroblasts strongly expressed CD73 and CD105, with a higher percentage of positive cells and stronger expression levels in GO. Neither cell type expresses CD14, CD19, and HLA-DR. Protein CD34 was expressed at low levels by 45% to 70% of the cells, with its expression significantly lower in GO cells. Graves' orbitopathy fibroblasts displayed features of osteogenesis (calcium deposits, and osteocalcin [BGLAP] and osteonectin [SPARC] expression), chondrogenesis (glycosaminoglycan production; SOX9 and aggrecan [ACAN] expression), myogenesis (alpha-smooth muscle actin expression), and neurogenesis (beta-III tubulin expression) upon differentiation. CONCLUSIONS: Our findings suggest that orbital fibroblasts contain a population of cells that fulfil the criteria defining MSC. This subpopulation may be increased in GO, possibly underlying the complex differentiation phenotype of the disease.

Krylova, T. A., et al. (2012). "[Comparative characteristics of new mesenchymal stem cell lines derived from human embryonic stem cells, bone marrow and foreskin]." Tsitologiia **54**(1): 5-16.

New nonimmortalized fibroblast-like cell lines SC5-MSC and SC3a-MSC, FetMSC, FRSN were obtained from human embryonic stem cells (ESC), bone marrow of a 5-6-days embryo and foreskin of a 3-years-old boy, respectively. All the lines are successfully used as the feeder at human ESC cultivation. It is determined that the average cell population doublings time varies from 25.5 h for ISC5-MSC to 38.8 h for SC3a-MSC. Active proliferation of all the lines is also shown by the corresponding growth curves. Numerical and structural karyotypic analysis showed that these lines had normal karyotype: 46,XX (SC5-MSC and SC3a-MSC) and 46,XY (FetMSC and FRSN). To determine the status of the lines, their cell surface markers were analyzed by flow cytometry. This analysis revealed the presence of surface antigens CD44, CD73, CD90, CD105 and HLA-ABC, characteristic of human MSC, and the absence of CD34 and HLA-DR. Different lines were found to express CD117(c-kit) to a different level. Immunofluorescence and flow cytometry analysis did not detect TRA-1-60 and Oct-4, characteristic of human embryonic stem cells, and revealed interlinear variations in the level of SSEA, which did not depend on the cell origin. It is not clear yet whether these interlinear variations affect functional MSC status. In all the lines, immunofluorescence

analysis showed the presence of the markers of early differentiation in the derivates of three germ layers which may allow MSC to be useful, in corresponding microenvironments, for reparation of tissue injures. Adipogenic and osteogenic differentiatiation of all cell lines has been shown.

Krylova, T. A., et al. (2014). "[Comparative characteristics of mesenchymal stem cell lines derived from bone marrow and muscle of limb of early human embryo]." Tsitologiia **56**(8): 562-573.

In this work, we have carried out a comparative analysis of the characteristics of mesenchymal stem cell lines isolated from different tissues of 5-6-weeks homan embryo: bone marrow (line FetMSC) and muscle of limb (line M-FetMSC). The basic characteristics of these lines were obtained at the 6th passage. Average population doubling time was 33.0 +/- 1.4 h (FetMSC) and 25.0 +/- 0.1 h (M-FetMSC). Growth curves also indicated active proliferation of cells of both lines. Numerical and structural karyotypic analysis showed that both lines have a normal karyotype: 46, XY. In order to determine the status of the lines, cell surface markers were analyzed by flow cytometry. The analysis revealed the presence of surface antigens specific for human MSCs, CD44, CD73, CD90, CD105, HLA-ABC, vimentin, and the lack of CD34 and HLA-DR, in both lines. The ability to differentiate into osteogenic, chondrogenic and adipogenic directions has been also shown for both lines. Immunofluorescence and flow cytometry analysis has detected no expression of the surface antigen TRA-1-60 in both lines, but has revealed high expression of the surface antigen SSEA-4 and low expression of transcription factor Oct-4 characteristic of human embryonic stem cells. In these lines, immunofluorescence analysis has shown the presence of the markers of early differentiation in the derivates of three germ layers characteristic of human embryonic stem cells, which provides significant opportunities for MSC to be useful, in corresponding microenvironments, for repair of tissue injures. Dispite confirming MSC status for FetMSC and M-FetMSC lines, a number of interlinear differences related to growth characteristics and differentiation potential were revealed. Adipogenic differentiatiation potential of M-FetMSC line was reduced compared with FetMSC line. Immunofluorescence analysis showed that, in the process of skeletal-muscle differentiation, Z-disks were revealed only in sarcomeres of M-FetMSC line. These findings suggest the possible influence of different microenvironments in which the cells are in the body before their transfer in vitro.

Krylova, T. A., et al. (2015). "[Characteristic of the Cellular Spheroids, Derived from Mesenchymal Stem Cell Lines from Bone Marrow and Muscle of Limb of Early Human Embryo]." Tsitologiia **57**(7): 480-490.

Cellular spheroids were derived from mesenchymal stem cell lines derived from 5-6-weeks embryo from different tissues of 5-6-week human embryo: bone marrow (FetMSC) and muscle of limb (M-FetMSC). Comparative analysis of the characteristics of these lines has been performed with 2D culturing in monolayer and 3D culturing in spheroids. The characteristics of cellular spheroids were obtained after 48 h after their formation from monolayer cultures on the 6th passage after decryopreservation. Spheroids in contrast to monolayer cultures are heterogeneous cell populations composed of fibroblast-like and epithelioid cells. Two-day spheroids are actively proliferating structure. Cell surface markers were analyzed using flow cytometry. Both in the monolayer cultures and cellular spheroids, this analysis has revealed the presence of expression of surface antigens CDD44, CD73, CD9O, CD105, HLA-ABC that are characteristic of human MSC, and the absence of expression if CD34 and HLA-DR. Nevertheless, the level of expression of CD90 and CD105 antigens was significantly lower in the spheroids as compared with corresponding monolayer cultures. Immunofluorescence and flow cytometry analysis of the expression of transcriptions factors and surface antigens characteristic of human embryonic stem cells showed the presence of expression of Sox-2 and SSEA-4 in 2D and 3D cultures. Lack of expression of Oct-4 in 2D cultures and its significant increase in 3D cultures has been found. Immunofluorescence analysis showed the presence of the markers of early differentiation in the derivates of three germ layers characteristic of human embryonic stem cells in the cellular spheroids of both lines, which coincides with 2D cultures of these lines. The directed osteogenic, chondrogenic and adipogenic differentiation of these lines has been shown. However, a number of differences has been found between

monolayer cultures and spheroids. Adipogenic differentiation was more active in the cellular spheroids from cell line M-FetMSC a compared with corresponding monolayer cultures. Differences between the 2D and 3D cultures of both lines have been shown by the character of chondrogenic differentiation. The results obtained confirm the status of MSC for the cellular spheroids derived from monolayer cultured of cell lines FetMSC and M-FetMSC and apparently indicate a partial extension of their differentiation capacity as compared to monolayer cultured.

Law, S. and S. Chaudhuri (2013). "Mesenchymal stem cell and regenerative medicine: regeneration versus immunomodulatory challenges." Am J Stem Cells **2**(1): 22-38.

Mesenchymal Stem cells (MSC) are now presented with the opportunities of multifunctional therapeutic approaches. Several reports are in support of their self-renewal, capacity for multipotent differentiation, and immunomodulatory properties. They are unique to contribute to the regeneration of mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, and adipose. In addition to promising trials in regenerative medicine, such as in the treatment of major bone defects and myocardial infarction, MSC has shown a therapeutic effect other than direct hematopoiesis support in hematopoietic reconstruction. MSCs are identified by the expression of many molecules including CD105 (SH2) and CD73(SH3/4) and are negative for the hematopoietic markers CD34, CD45, and CD14. Manufacturing of MSC for clinical trials is also an important aspect as their differentiation, homing and Immunomodulatory properties may differ. Their suppressive effects on immune cells, including T cells, B cells, NK cells and DC cells, suggest MSCs as a novel therapy for GVHD and other autoimmune disorders. Since the cells by themselves are non-immunogenic, tissue matching between MSC donor and recipient is not essential and, MSC may be the first cell type able to be used as an "off-the-shelf" therapeutic product. Following a successful transplantation, the migration of MSC to the site of injury refers to the involvement of chemokines and chemokine receptors of respective specificity. It has been demonstrated that cultured MSCs have the ability to engraft into healthy as well as injured tissue and can differentiate into several cell types in vivo, which facilitates MSC to be an ideal tool for regenerative therapy in different disease types. However, some observations have raised questions about the limitations for proper use of MSC considering some critical factors that warn regular clinical use.

Lee, H. R., et al. (2014). "Effects of octylphenol on the expression of cell cycle-related genes and the growth of mesenchymal stem cells derived from human umbilical cord blood." Int J Mol Med **33**(1): 221-226.

Umbilical cord blood (UCB) is defined as blood that exists in the placenta and in the attached umbilical cord following childbirth. Cord blood is now used for research purposes as it contains mesenchymal stem cells (MSCs), multipotent stromal cells which have the ability to differentiate into a variety of cell types. Among endocrine disrupting chemicals (EDCs), octylphenol (OP) is one of the alkylphenols, which are widely used industrial chemicals; these chemicals cause a number of serious side-effects, such as reproductive abnormalities. In this study, we isolated human MSCs from UCB and demonstrate that cultured MSCs express the surface marker, CD34, but not CD105. We further examined the effects of OP on human UCB-derived MSCs following exposure to OP by cell proliferation assay, semi-quantitative RT-PCR and western blot analysis. The results revealed that the transcriptional and translational levels of cyclin D1 were increased, while the levels of p21 were suppressed in the MSCs treated with OP compared with the negative controls. This collapse of the regulation of the cell cycle may directly stimulate the growth of the MSCs under culture conditions. The results from the present study provide further insight into the effects of common EDCs on MSCs derived from human UCB. However, further studies are required to identify the signaling pathways which mediate the effects of EDCs on MSCs.

Lee, I. C., et al. (2007). "Development of a useful technique to discriminate anterior cruciate ligament cells and mesenchymal stem cells--the application of cell electrophoresis." J Biomed Mater Res A **82**(1): 230-237.

Mesenchymal stem cells (MSCs) can differentiate into multiple nonhematopoietic cell lineages, including osteoblasts, chondrocytes, and ligament cells. The purpose

of this study is to identify the difference between MSCs and anterior cruciate ligament (ACL) cells for the application of distinguishing these two cells during the process of MSCs differentiating into ACL cells. Although culture of MSCs and ACL cells have been studied extensively, it was found that these two cells could not be distinguished from their appearance, expression of surface antigens (including CD105, CD34, CD45, CD29, CD44, and CD71), alpha-smooth muscle actin, and mRNAs for type I collagen, type III collagen, and tenascin-C, based on a series of traditional methods for cell identification. Cell electrophoresis, measuring the electrophoretic mobility (EPM) of cells, was proposed to investigate the discrepancy in surface charge properties of MSCs and ACL cells. Surprisingly, the EPM value of MSCs is significantly greater than that of ACL cells ($p < 0.001$). Although cell electrophoresis cannot determine the specific surface protein, it can reflect the net surface charge density of cell membrane, which can be influenced by the dissociation of functional groups of peripheral membrane proteins. Therefore, it is suggested that cell electrophoresis, while simple and cheap in manipulation, can serve as a useful research tool to assist in identification of MSCs differentiating into ACL cells.

Lei, M., et al. (2014). "Mesenchymal stem cell characteristics of dental pulp and periodontal ligament stem cells after in vivo transplantation." Biomaterials **35**(24): 6332-6343.

Mesenchymal stem cells (MSCs) isolated from human postnatal dental pulp and periodontal ligament (PDL) tissues can give rise to multilineage differentiation in vitro and generate related dental tissues in vivo. However, the cell properties of human dental pulp stem cells (DPSCs) and PDL stem cells (PDLSCs) after in vivo implantation remain largely unidentified. In this study, cells were re-isolated from in vivo-generated dental pulp-like and PDL-like tissues (termed re-DPCs and re-PDLCs, respectively) as a result of ectopic transplantation of human DPSC and PDLSC sheets. The cell characteristics in terms of colony-forming ability, cell surface antigens and multi-differentiation potentials were all evaluated before and after implantation. It was found that re-DPCs and re-PDLCs were of human and mesenchymal origin and positive for MSC markers such as STRO-1, CD146, CD29, CD90 and CD105; and, to some extent, re-DPCs could maintain their colony forming abilities. Moreover, both cell types were able to form mineral deposits and differentiate into adipocytes and chondrocytes; however, quantitative analysis and related gene expression determination showed that the osteo-/chondro-differentiation capabilities of re-DPCs and re-PDLCs were significantly reduced compared to those of DPSCs and PDLSCs, respectively ($P < 0.05$); re-PDLCs showed a greater reduction potential than re-DPCs. We conclude that DPSCs and PDLSCs may maintain their MSC characteristics after in vivo implantation and, compared to PDLSCs, DPSCs appear much more stable under in vivo conditions. These findings provide additional cellular and molecular evidence that supports expanding the use of dental tissue-derived stem cells in cell therapy and tissue engineering.

Leyva-Leyva, M., et al. (2013). "Characterization of mesenchymal stem cell subpopulations from human amniotic membrane with dissimilar osteoblastic potential." Stem Cells Dev **22**(8): 1275-1287.

Human fetal mesenchymal stem cells can be isolated from the amniotic membrane (AM-hMSCs) by enzymatic digestion. The biological properties of this cell population have been characterized; however, few studies have focused on the presence of stem cell subpopulations and their differentiation potential. The aim of the present study was to isolate homogeneous AM-hMSC subpopulations based on the coexpression of surface markers. In addition, we aimed to characterize stem cell subpopulations through the detection of typical stem cell markers and its differentiation potential. In this study, fluorescence-activated cell sorting (FACS) was used to positively select for the surface markers CD44, CD73, and CD105. Two subpopulations were isolated: CD44+ / CD73+ / CD105+ (CD105+), and CD44+ / CD73+ / CD105- (CD105-). To characterize the cell subpopulations, the expression of pluripotency-associated markers was analyzed by reverse transcriptase-polymerase chain reaction and immunofluorescence. Our results showed positive expression of SOX2, SOX3, PAX6, OCT3/4, and NANOG in the CD105+ and CD105(-) cell subpopulations. In contrast, we did not detect expression of SSEA4 or FOXD3 in either subpopulation. Immunophenotypes, such as mesenchymal

and hematopoietic markers, were studied by FACS analyses. Our data revealed the expression of the CD49a, CD49d, CD29, integrin alpha9beta1, CD44, CD73, and CD105 antigens in both subpopulations. In contrast, CD90, CD45, CD34, CD14, and HLA-DR expression was not detected. The ability of both subpopulations to differentiate into osteoblasts, adipocytes, and chondrocytes was evidenced using Alizarin red, Oil-Red, and Alcian blue staining, respectively. Furthermore, neuronal differentiation was demonstrated by the expression of GFAP and NEURO-D. Interestingly, we observed a dissimilar osteoblastic differentiation potential between the subpopulations. CD105- cells showed stronger expression of secreted protein acidic and rich in cysteine (SPARC) and osteonectin, which was associated with more effective calcium deposition, than CD105+ cells. In conclusion, we described a systematic method for the isolation of hMSCs that was highly reproducible and generated homogeneous cultures for osteoblast differentiation with an efficient capacity for mineralization.

Li, F. and C. Niyibizi (2016). "Engraftability of Murine Bone Marrow-Derived Multipotent Mesenchymal Stem Cell Subpopulations in the Tissues of Developing Mice following Systemic Transplantation." Cells Tissues Organs **201**(1): 14-25.

INTRODUCTION: Cell therapies for generalized musculoskeletal diseases would require distribution of cells to all the skeletal tissues; however, there are controversies regarding the transplantability of multipotent mesenchymal stems cells (MSCs). We generated single-cell subpopulations of MSCs from murine bone marrow and assessed them for differences in trafficking through the circulatory system and engraftment in bone and other tissues. MATERIALS AND METHODS: Seven single-cell clonal subpopulations were generated by serial dilution of GFP-marked MSCs isolated from bone marrow. The subpopulations were examined for putative MSC surface marker expression, in vitro differentiation toward osteogenic and adipogenic lineages, migration and engraftment in different tissues following intravenous delivery in normal, sublethally irradiated neonatal mice. RESULTS: The surface marker expression profile revealed notable differences among clonal cells, specifically CD44 and CD105. All the cell subpopulations differentiated toward osteogenic and adipogenic lineages, with some committed to only one or the other. Two clones enriched in CXCR4 expression were highly efficient in migrating and engrafting in skeletal tissue including bone; this confirmed the role of this chemokine in cell migration. Donor cells retrieved from various tissues displayed different morphologies and potential differentiation into tissue cell type of engraftment, suggesting modification by the tissues in which the donor cells engrafted. CONCLUSION: We have reported that, within bone marrow, there are heterogeneous subpopulations of MSCs that may differ in their ability to migrate in the circulatory system and engraft in different tissues.

Li, N., et al. (2017). "Lysophosphatidic acid enhances human umbilical cord mesenchymal stem cell viability without differentiation via LPA receptor mediating manner." Apoptosis **22**(10): 1296-1309.

Human umbilical cord mesenchymal stem cells (hUC-MSCs) are potential stromal cells which are regarded as the most feasible stem cell group in cell therapy. The maintenance of cell survival without differentiation is important in cell transplantation and stem cell therapy. However, negative factors exist in cell transplantation. Lysophosphatidic acid (LPA) is a non-antigenic small molecule phospholipid which induced several fundamental cellular responses, such as cell proliferation, apoptosis and migration. In this study we aimed to explore the effects of LPA on the survival and differentiation of MSCs and its availability in cell therapy. We found that LPA stimulated hUC-MSC proliferation and protected hUC-MSCs from lipopolysaccharide (LPS) induced apoptosis. We also observed that CD29, CD44, CD73, CD90 and CD105 were expressed, whereas CD34 and CD45 were not expressed in hUC-MSCs, and these makers have no change in LPA containing medium, which indicated that LPA accelerated the survival of hUC-MSCs in an undifferentiating status. We also demonstrated that higher expressed LPAR1 involved in LPA stimulated cell survival action. LPA stimulated cell proliferation was associated with LPAR1 mediated Gi/o-proteins/ERK1/2 pathway. On the other hand, LPA protected hUC-MSCs from LPS-induced apoptosis through suppressing caspase-3 activation by LPAR1 coupled with a G protein, but not Gi/o or Gq/11 in hUC-MSC.

Collectively, this study demonstrated that LPA increased the proliferation and survival of hUC-MSCs without differentiation through LPAR1 mediated manner. Our findings provide that LPA as a anti-apoptotic agent having potential application prospect in cell transplantation and stem cell therapy.

Li, O., et al. (2013). "Human embryonic stem cell-derived mesenchymal stroma cells (hES-MSCs) engraft in vivo and support hematopoiesis without suppressing immune function: implications for off-the shelf ES-MSC therapies." PLoS One **8**(1): e55319.

Mesenchymal stroma cells (MSCs) have a high potential for novel cell therapy approaches in clinical transplantation. Commonly used bone marrow-derived MSCs (BM-MSCs), however, have a restricted proliferative capacity and cultures are difficult to standardize. Recently developed human embryonic stem cell-derived mesenchymal stroma cells (hES-MSCs) might represent an alternative and unlimited source of hMSCs. We therefore compared human ES-cell-derived MSCs (hES-MP002.5 cells) to normal human bone marrow-derived MSCs (BM-MSCs). hES-MP002.5 cells had lower yet reasonable CFU-F capacity compared with BM-MSC (8+/-3 versus 29+/-13 CFU-F per 100 cells). Both cell types showed similar immunophenotypic properties, i.e. cells were positive for CD105, CD73, CD166, HLA-ABC, CD44, CD146, CD90, and negative for CD45, CD34, CD14, CD31, CD117, CD19, CD 271, SSEA-4 and HLA-DR. hES-MP002.5 cells, like BM-MSCs, could be differentiated into adipocytes, osteoblasts and chondrocytes in vitro. Neither hES-MP002.5 cells nor BM-MSCs homed to the bone marrow of immune-deficient NSG mice following intravenous transplantation, whereas intra-femoral transplantation into NSG mice resulted in engraftment for both cell types. In vitro long-term culture-initiating cell assays and in vivo co-transplantation experiments with cord blood CD34+ hematopoietic cells demonstrated furthermore that hES-MP002.5 cells, like BM-MSCs, possess potent stroma support function. In contrast to BM-MSCs, however, hES-MP002.5 cells showed no or only little activity in mixed lymphocyte cultures and phytohemagglutinin (PHA) lymphocyte stimulation assays. In summary, ES-cell derived MSCs might be an attractive unlimited source for stroma transplantation approaches without suppressing immune function.

Liao, J., et al. (2011). "Cells isolated from inflamed periapical tissue express mesenchymal stem cell markers and are highly osteogenic." J Endod **37**(9): 1217-1224.

INTRODUCTION: We previously reported the presence of mesenchymal stem/progenitor cells (MSCs) in inflamed pulp tissue. Here we asked whether MSCs also exist in inflamed periapical tissues resulting from endodontic infection. The objectives of this study were to detect the expression of MSC markers in periapical inflammatory tissues and to characterize isolated cells from these tissues. METHODS: Human periapical inflammatory tissues were collected and processed to detect MSC marker expression by immunohistochemistry. Cells were isolated and tested for cell surface marker expression by using flow cytometry and examined for multiple differentiation potential into osteogenic and adipogenic pathways. In vivo formation of mineralized tissues was assessed in a mouse model. RESULTS: Immunohistochemistry showed positive staining for MSC markers STRO-1, CD90, and CD146. Isolated cells at passage 0 appeared as typical fibroblastic cells, and a few cells formed colony-forming unit-fibroblasts (CFU-Fs). After passaging, the CFU-F forming ability diminished dramatically, and the population doubling was up to 26. Flow cytometry data showed that these cells at passage 2 expressed low levels of STRO-1 and CD146 and moderate to high levels of CD90, CD73, and CD105. At passage 6, the levels of these markers decreased. When incubated in specific differentiation medium, cells demonstrated a strong osteogenic but weak adipogenic capacity. After in vivo cell transplantation, mineralized tissues formed in immunocompromised mice. CONCLUSIONS: Human periapical inflammatory tissues expressed MSC markers, suggesting the presence of MSCs. Isolated cells exhibited typical mesenchymal cell immunophenotype with a capacity to form mineralized matrix in vitro and in vivo.

Lin, C. S., et al. (2013). "Commonly used mesenchymal stem cell markers and tracking labels: Limitations and challenges." Histol Histopathol **28**(9): 1109-1116.

Early observations that cultured mesenchymal stem cells (MSCs) could be induced to exhibit certain characteristics of osteocytes and chondrocytes led to the proposal that they could be transplanted for tissue repair through cellular differentiation. Therefore, many subsequent preclinical studies with transplanted MSCs have strived to demonstrate that cellular differentiation was the underlying mechanism for the therapeutic effect. These studies generally followed the minimal criteria set by The International Society for Cellular Therapy in assuring MSC identity by using CD70, CD90, and CD105 as positive markers and CD34 as a negative marker. However, the three positive markers are co-expressed in a wide variety of cells, and therefore, even when used in combination, they are certainly incapable of identifying MSCs in vivo. Another frequently used MSC marker, Stro-1, has been shown to be an endothelial antigen and whether it can identify MSCs in vivo remains unknown. On the other hand, the proposed negative marker CD34 has increasingly been shown to be expressed in native MSCs, such as in the adipose tissue. It has also helped establish that MSCs are likely vascular stem cells (VSCs) that reside in the capillaries and in the adventitia of larger blood vessels. These cells do not express CD31, CD104b, or alpha-SMA, and therefore are designated as CD34+CD31-CD140b-SMA-. Many preclinical MSC transplantation studies have also attempted to demonstrate cellular differentiation by using labeled MSCs. However, all commonly used labels have shortcomings that often complicate data interpretation. The beta-gal (LacZ) gene as a label is problematic because many mammalian tissues have endogenous beta-gal activities. The GFP gene is similarly problematic because many mammalian tissues are endogenously fluorescent. The cell membrane label DiI can be adsorbed by host cells, and nuclear stains Hoechst dyes and DAPI can be transferred to host cells. Thymidine analog BrdU is associated with loss of cellular protein antigenicity due to harsh histological conditions. Newer thymidine analog EdU is easier to detect by chemical reaction to azide-conjugated Alexa fluors, but certain bone marrow cells are reactive to these fluors in the absence of EdU. These caveats need to be taken into consideration when designing or interpreting MSC transplantation experiments.

Lin, J. J., et al. (2014). "Malignant phyllodes tumors display mesenchymal stem cell features and aldehyde dehydrogenase/disialoganglioside identify their tumor stem cells." Breast Cancer Res **16**(2): R29.

INTRODUCTION: Although breast phyllodes tumors are rare, there is no effective therapy other than surgery. Little is known about their tumor biology. A malignant phyllodes tumor contains heterologous stromal elements, and can transform into rhabdomyosarcoma, liposarcoma and osteosarcoma. These versatile properties prompted us to explore their possible relationship to mesenchymal stem cells (MSCs) and to search for the presence of cancer stem cells (CSCs) in phyllodes tumors. METHODS: Paraffin sections of malignant phyllodes tumors were examined for various markers by immunohistochemical staining. Xenografts of human primary phyllodes tumors were established by injecting freshly isolated tumor cells into the mammary fat pad of non-obese diabetic-severe combined immunodeficient (NOD-SCID) mice. To search for CSCs, xenografted tumor cells were sorted into various subpopulations by flow cytometry and examined for their in vitro mammosphere forming capacity, in vivo tumorigenicity in NOD-SCID mice and their ability to undergo differentiation. RESULTS: Immunohistochemical analysis revealed the expression of the following 10 markers: CD44, CD29, CD106, CD166, CD105, CD90, disialoganglioside (GD2), CD117, Aldehyde dehydrogenase 1 (ALDH), and Oct-4, and 7 clinically relevant markers (CD10, CD34, p53, p63, Ki-67, Bcl-2, vimentin, and Globo H) in all 51 malignant phyllodes tumors examined, albeit to different extents. Four xenografts were successfully established from human primary phyllodes tumors. In vitro, ALDH+ cells sorted from xenografts displayed approximately 10-fold greater mammosphere-forming capacity than ALDH- cells. GD2+ cells showed a 3.9-fold greater capacity than GD2- cells. ALDH+/GD2+cells displayed 12.8-fold greater mammosphere forming ability than ALDH-/GD2- cells. In vivo, the tumor-initiating frequency of ALDH+/GD2+ cells were up to 33-fold higher than that of ALDH+ cells, with as few as 50 ALDH+/GD2+ cells being sufficient for engraftment. Moreover, we provided the first evidence for the induction of ALDH+/GD2+ cells to differentiate into neural cells of

various lineages, along with the observation of neural differentiation in clinical specimens and xenografts of malignant phyllodes tumors. ALDH+ or ALDH+/GD2+ cells could also be induced to differentiate into adipocytes, osteocytes or chondrocytes. CONCLUSIONS: Our findings revealed that malignant phyllodes tumors possessed many characteristics of MSC, and their CSCs were enriched in ALDH+ and ALDH+/GD2+ subpopulations.

Lin, T. C., et al. (2017). "Pueraria mirifica inhibits 17beta-estradiol-induced cell proliferation of human endometrial mesenchymal stem cells." Taiwan J Obstet Gynecol **56**(6): 765-769.

OBJECTIVE: The notion that the human endometrium may contain a population of stem cells has recently been proposed. The mesenchymal stem cells (MSCs) in the endometrium are believed to be responsible for the remarkable regenerative ability of endometrial cells. Estrogens influence the physiological and pathological processes of several hormone-dependent tissues, such as the endometrium. Pueraria mirifica (PM) is a herbal plant that contains several phytoestrogens, including isoflavones, lignans, and coumestans, and is known to exert an estrogenic effect on animal models. The present study investigated the effects of PM on the proliferation of human endometrial MSCs (hEN-MSCs). MATERIALS AND METHODS: The hEN-MSCs were isolated from human endometrial tissue. The surface markers of these hEN-MSCs were identified through reverse transcription-polymerase chain reaction analysis. The proliferation potential of hEN-MSCs was measured through a cell proliferation assay. Multilineage differentiation ability was confirmed through Oil red O and von Kossa staining. RESULTS: This study demonstrated that 17beta-estradiol-responsive MSCs with Oct-4, CD90, and CD105 gene expression can be derived from the human endometrium and that PM exerts biological effects on hEN-MSCs, specifically, enhanced cell growth rate, through the estrogen receptor. Furthermore, PM at 1500 and 2000 mug/mL significantly increased cell proliferation compared with the vehicle control, and PM concentration at 1000 mug/mL significantly inhibited the enhanced cell growth rate induced by 17beta-estradiol in hEN-MSCs. CONCLUSION: This study provides new insights into the possible biological effects of PM on the proliferation of hEN-MSCs.

Liotta, F., et al. (2015). "Mesenchymal stem cells are enriched in head neck squamous cell carcinoma, correlates with tumour size and inhibit T-cell proliferation." Br J Cancer **112**(4): 745-754.

BACKGROUND: Cancer is a multifactorial disease not only restricted to transformed epithelium, but also involving cells of the immune system and cells of mesenchymal origin, particularly mesenchymal stem cells (MSCs). Mesenchymal stem cells contribute to blood- and lymph- neoangiogenesis, generate myofibroblasts, with pro-invasive activity and may suppress anti-tumour immunity. METHODS: In this paper, we evaluated the presence and features of MSCs isolated from human head neck squamous cell carcinoma (HNSCC). RESULTS: Fresh specimens of HNSCC showed higher proportions of CD90+ cells compared with normal tissue; these cells co-expressed CD29, CD105, and CD73, but not CD31, CD45, CD133, and human epithelial antigen similarly to bone marrow-derived MSCs (BM-MSCs). Adherent stromal cells isolated from tumour shared also differentiation potential with BM-MSCs, thus we named them as tumour-MSCs. Interestingly, tumour-MSCs showed a clear immunosuppressive activity on in vitro stimulated T lymphocytes, mainly mediated by indoelamine 2,3 dioxygenase activity, like BM-MSCs. To evaluate their possible role in tumour growth in vivo, we correlated tumour-MSC proportions with neoplasm size. Tumour-MSCs frequency directly correlated with tumour volume and inversely with the frequency of tumour-infiltrating leukocytes. CONCLUSIONS: These data support the concept that tumour-MSCs may favour tumour growth not only through their effect on stromal development, but also by inhibiting the anti-tumour immune response.

Liu, Y., et al. (2012). "One-step derivation of mesenchymal stem cell (MSC)-like cells from human pluripotent stem cells on a fibrillar collagen coating." PLoS One **7**(3): e33225.

Controlled differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) into cells that resemble adult mesenchymal stem cells (MSCs) is an attractive approach to obtain a readily available source of progenitor cells for

tissue engineering. The present study reports a new method to rapidly derive MSC-like cells from hESCs and hiPSCs, in one step, based on culturing the cells on thin, fibrillar, type I collagen coatings that mimic the structure of physiological collagen. Human H9 ESCs and HDFa-YK26 iPSCs were singly dissociated in the presence of ROCK inhibitor Y-27632, plated onto fibrillar collagen coated plates and cultured in alpha minimum essential medium (alpha-MEM) supplemented with 10% fetal bovine serum, 50 uM magnesium L-ascorbic acid phosphate and 100 nM dexamethasone. While fewer cells attached on the collagen surface initially than standard tissue culture plastic, after culturing for 10 days, resilient colonies of homogenous spindle-shaped cells were obtained. Flow cytometric analysis showed that a high percentage of the derived cells expressed typical MSC surface markers including CD73, CD90, CD105, CD146 and CD166 and were negative as expected for hematopoietic markers CD34 and CD45. The MSC-like cells derived from pluripotent cells were successfully differentiated in vitro into three different lineages: osteogenic, chondrogenic, and adipogenic. Both H9 hES and YK26 iPS cells displayed similar morphological changes during the derivation process and yielded MSC-like cells with similar properties. In conclusion, this study demonstrates that bioimimetic, fibrillar, type I collagen coatings applied to cell culture plates can be used to guide a rapid, efficient derivation of MSC-like cells from both human ES and iPS cells.

Lu, J. M., et al. (2010). "A preliminary study of mesenchymal stem cell-like cells derived from murine corneal stroma." Graefes Arch Clin Exp Ophthalmol **248**(9): 1279-1285.

BACKGROUND: Mesenchymal stem cells can be isolated from various tissues besides bone marrow and can differentiate into cells of three germ layers. Recent studies indicate that some cells in corneal stroma express stem cell markers and can also differentiate into chondrocytes and neurocytes. This study was carried out to investigate whether mesenchymal stem cells reside in the murine corneal stroma. METHODS: Corneas of BALB/c mice were treated with collagenase digestion after the epithelium and endothelium were removed. Then the single cells were harvested and further identified by reverse transcription polymerase chain reaction (RT-PCR). After the immunophenotype of passage 2 corneal stroma-derived cells was analyzed by flow cytometry, attempts were made to differentiate these cells into adipocytes and osteocytes using conditioned medium. Following induction, cells were evaluated by RT-PCR, oil red O and Alizarin Red staining. RESULTS: Isolated single cells were of stromal origin, not of epithelial or endothelial. Passage 2 corneal stroma-derived cells exhibited the spindle-shaped morphology and expressed CD29, CD90, CD105, and CD71; but were negative for CD34 and CD45. In addition, these cells showed the potentiality of differentiating into adipocytes and osteocytes, which was confirmed by RT-PCR and staining. CONCLUSION: This study demonstrates the presence of mesenchymal stem cell-like cells in the murine corneal stroma. Further analysis of these cells will aid elucidation of the mechanisms of some keratopathies, and these cells may be a source for bioengineering of corneal tissue and for cell-based therapeutics.

Lu, S. H., et al. (2018). "[Effect of CD106(+) Mesenchymal Stem Cell on Bone Marrow Vascular Failure in Patients with Aplastic Anemia]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao **40**(2): 178-186.

Objective To investigate the vascularization ability of mesenchymal stem cells(MSCs)and explore its influencing factors in aplastic anemia(AA) patients. Methods MSCs were isolated from the bone marrow of AA patients(AA MSCs) and normal controls(N MSCs) were cultured and then evaluated by flow cytometry and immunofluorescene staining technique.The expression level of vascular cell adhesion molecule-1(CD106) was detected by gene sequencing,and the content and fluorescene intensity of CD106(+)MSCs was determined by fluorescence-activated cell sorting.The content of CD105(+)CD106(+)MSCs in fresh AA bone marrow was measured,followed by the determination of the capability of endothelial differentiation from AA MSCs and N MSCs with immunofluorescene analysis;finally,the capability of CD31(+)cell differentiation from CD106-blocking N MSCs and its tubular structures formation in matrigel were tested.Results The expression of CD106 in AA patients was defective(decreased by 12.13 times when compared with N MSCs) and the concentration and fluorescene degree of CD106(+)MSCs was also decreased in AA patients [(28.03+/-17.71)% vs.(59.61+/-

12.26)%,P=0.000].The content of CD105(+)CD106(+)MSCs decreased significantly in the fresh bone marrow [(0.33+/-0.10)% vs.(2.98+/-0.46)%,P=0.0005].Besides, the capability of CD31(+)cell differentiation from AA MSCs was significantly delayed [(13.67+/-1.50)% vs.(43.24+/-0.96)%,P=0.0004].Also,the capability of CD31(+)cell differentiation and tubular structures formation of CD106-blocking N MSCs was also obviously decreased [(26.00+/-2.65)% vs.(91.78+/-2.44)%,P=0.000;(13.81+/-1.98)mm vs.(68.12+/-6.78)mm,P=0.0015].Conclusion The deficient or decreased expression of CD106(+)MSCs accelerate the bone marrow vascularization failure in AA patients.

Lu, Z. Y., et al. (2013). "[TNF-alpha stimulates bone marrow mesenchymal stem cell VCAM-1 production by ERK signaling pathway]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **21**(6): 1568-1571.

This study was aimed to explore the effect of TNF-alpha on the vascular cell adhesion molecule 1 (VCAM-1) expression of human bone marrow mesenchymal stem cells (BMMSC) and the relationship between this process and ERK signalling pathway. BMMSC were isolated by density gradient centrifugation combined with adherent culture method, and then identified by surface antigen expression and differentiation potential. Flow cytometry was used to detect expression of VCAM-1 on BMMSC exposed to TNF-alpha at different concentrations, and the effect of ERK inhibitor U0126 on VCAM-1 of BMMSC. ERK signaling pathway activation was analyzed by Western blot. The results showed that BMMSC positively expressed CD29, CD69, CD44, CD105, and negatively expressed CD34, CD45. BMMSC could be induced to differentiate into osteoblasts and adipocytes. Flow cytometry analysis showed that after the TNF-alpha stimulation, the expression of VCAM-1 on BMMSC increased in a dose-dependent manner. And this increase was inhibited by U0126. TNF-alpha caused activation of ERK signal pathway, and U0126 suppressed this effect induced by TNF-alpha. It is concluded that TNF-alpha can increase expression of VCAM-1 of BMMSC via ERK signaling pathway.

Luznik, Z., et al. (2016). "Effect of Cryopreserved Amniotic Membrane Orientation on the Expression of Limbal Mesenchymal and Epithelial Stem Cell Markers in Prolonged Limbal Explant Cultures." PLoS One **11**(10): e0164408.

PURPOSE: To evaluate the effect of prolonged limbal explants cultured without any scaffolds or on amniotic membrane (AM) on the viability, proliferation and differentiation potential of putative phenotypically defined cultured limbal mesenchymal (LMSC) and epithelial stem cells (LESC). METHODS: Limbal explants were cultivated on cryopreserved intact AM or plastic plates using medium supplemented with only human serum. AM was positioned with either the epithelial or stromal side up. The outgrowing cells were immunophenotyped for the co-expression of mesenchymal stem cell markers (CD73/CD90/CD105 positive and CD45 negative), proliferation and putative progenitor markers (CXCR4, CD117), epithelial markers and antigen presenting cell markers (CD80, CD83, CD86) by flow cytometry. Immunohistochemistry on limbal cultures cultivated on AM was carried out with antibodies against pan-cytokeratin, p63, Ki67. RESULTS: Morphological and immunostaining analyses revealed two distinct stem cell population types, which could be identified over prolonged culturing time periods. Expression of LMSC markers and CXCR4 was significantly higher ($p < 0.05$) in cultures cultivated without AM. However, no statistically significant difference was observed in CD117 expression. The cells cultivated on AM retained an epithelial cell structure, which was further confirmed by histology examination. Histology revealed limbal epithelial growth and p63, Ki67 positive cells on both sides of AM. CONCLUSION: Limbal cells cultivated on AM exhibited a lower expression profile of LMSC and CXCR4 markers as limbal cells cultivated on plastic culture plates. However, CD117 expression was similar. Histology confirmed limbal epithelial cell growth on both sides of AM, with no morphological differences, or positivity of cells for p63 and Ki67.

Majore, I., et al. (2009). "Identification of subpopulations in mesenchymal stem cell-like cultures from human umbilical cord." Cell Commun Signal **7**: 6.

BACKGROUND: A variety of cell types can be identified in the adherent fraction of bone marrow mononuclear cells including more primitive and embryonic-like stem cells,

mesenchymal stem cells (MSC), lineage-committed progenitors as well as mature cells such as osteoblasts and fibroblasts. Different methods are described for the isolation of single bone marrow stem cell subpopulations - beginning from ordinary size sieving, long term cultivation under specific conditions to FACS-based approaches. Besides bone marrow-derived subpopulations, also other tissues including human umbilical cord (UC) have been recently suggested to provide a potential source for MSC. Although of clinical importance, these UC-derived MSC populations remain to be characterized. It was thus the aim of the present study to identify possible subpopulations in cultures of MSC-like cells obtained from UC. We used counterflow centrifugal elutriation (CCE) as a novel strategy to successfully address this question. RESULTS: UC-derived primary cells were separated by CCE and revealed differentially-sized populations in the fractions. Thus, a subpopulation with an average diameter of about 11 mum and a small flat cell body was compared to a large sized subpopulation of about 19 mum average diameter. Flow cytometric analysis revealed the expression of certain MSC stem cell markers including CD44, CD73, CD90 and CD105, respectively, although these markers were expressed at higher levels in the small-sized population. Moreover, this small-sized subpopulation exhibited a higher proliferative capacity as compared to the total UC-derived primary cultures and the large-sized cells and demonstrated a reduced amount of aging cells. CONCLUSION: Using the CCE technique, we were the first to demonstrate a subpopulation of small-sized UC-derived primary cells carrying MSC-like characteristics according to the presence of various mesenchymal stem cell markers. This is also supported by the high proliferative capacity of these MSC-like cells as compared to whole primary culture or other UC-derived subpopulations. The accumulation of a self-renewing MSC-like subpopulation by CCE with low expression levels of the aging marker senescence-associated beta-galactosidase provides a valuable tool in the regenerative medicine and an alternative to bone-marrow-derived MSC.

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.

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.

Martin, J., et al. (2008). "Adult lung side population cells have mesenchymal stem cell potential." Cytotherapy **10**(2): 140-151.

BACKGROUND: The development of stem cell therapy for pulmonary diseases remains a challenge. Many diverse cell types reside within the lung and a common stem cell has not yet been identified. A basic understanding of lung stem cell fate during disease may prove important for drug intervention as well as autologous therapies. Niches for resident mesenchymal stem cells (MSC) have been identified in many adult tissues and more recently in the lung. We present data to confirm the observation that non-hematopoietic CD45(neg) lung side population (SP) cells contain MSC, single cells capable of multilineage differentiation. METHODS We carried these observations forward by analyzing the MSC potential of single-cell clones, as well as their chromosomal stability and telomerase activity. RESULTS: The expression of MSC markers was characterized in mouse CD45(neg) lung SP by flow cytometry on freshly isolated or cultured clonal populations. The karyotype of these cells was subsequently assayed by banding analysis, and telomerase activity was assessed using quantitative polymerase chain reaction. MSC differentiation potential was confirmed by the characteristic ability of single-cell clones to differentiate into cells of three mesenchymal lineages, chondrocytes, adipocytes and osteocytes. Differentiation was confirmed by histochemical analysis. All analyzed populations of CD45(neg) lung SP expressed mesenchymal markers (CD44, CD90, CD105, CD106, CD73 and Sca-I) and lacked hematopoietic markers (CD45, c-kit, CD11b, CD34 and CD14). The cultured and clonal CD45(neg) lung SP had normal chromosomal structures and expressed high levels of telomerase. After being expanded and cultured in differentiation medium, all populations of CD45(neg) lung SP demonstrated adipogenic, osteogenic and chrondrogenic potential. Adult CD45(neg) lung SP cells are a source of MSC. DISCUSSION: In defining this tissue-specific stem cell population in the lung, we are now better able to clarify a potential role for them in lung diseases.

Mazaheri, Z., et al. (2011). "Different doses of bone morphogenetic protein 4 promote the expression of early germ cell-specific gene in bone marrow mesenchymal stem cells." In Vitro Cell Dev Biol Anim **47**(8): 521-525.

Bone morphogenetic protein (BMP)-4 has a crucial role on primordial germ cells (PGCs) development in vivo which can promote stem cell differentiation to PG-like cells. In this study, we investigated the expression of Mvh as one of the specific genes in primordial germ cells after treatment with different doses of BMP4 on bone mesenchymal stem cells (BMSCs)-derived PGCs. Following isolation of BMSCs from male mouse femur and tibia, cells were cultured in medium for 72 h. Passage 4 murine BMSCs were characterized by CD90, CD105, CD34, and CD45 markers and osteo-adipogenic differentiation. Different doses of BMP4 (0, 0.01, 0.1, 1, 5, 25, 50, and 100 ng/ml) were added to BMSCs for PGCs differentiation during 4-days culture. Viability percent, proliferation rates, and expression of Mvh gene were analyzed by RT-qPCR. Data analysis was done with ANOVA test. CD90(+), CD105(+), CD34(-), and CD45(-) BMSCs were able to differentiate to osteo-adipogenic lineages. The results revealed that proliferation rate and viability percent were raised significantly ($p \leq 0.05$) by adding 1, 5, 25 ng/ml of BMP4 and there were decreased to the lowest rate after adding 100 ng/ml BMP4 ($p \leq 0.05$). There were significant up regulation ($p \leq 0.05$) in Mvh expression between 25, 50, and 100 ng/ml BMP4 with other doses. So the selective dose of BMP-4 for treatment during 4-day culture was 25 ng/ml. The results suggest that addition of 25 ng/ml BMP4 had the best effects based on gene-specific marker expression.

Moslem, M., et al. (2015). "Mesenchymal Stem/Stromal Cells Derived from Induced Pluripotent Stem Cells Support CD34(pos) Hematopoietic Stem Cell Propagation and Suppress Inflammatory Reaction." Stem Cells Int **2015**: 843058.

Mesenchymal stem/stromal cells (MSCs) represent a promising cell source for research and therapeutic applications, but their restricted ex vivo propagation capabilities limit putative applications. Substantial self-renewing of stem cells can be achieved by reprogramming cells into induced pluripotent stem cells (iPSCs) that can be easily expanded as undifferentiated cells even in mass culture. Here, we investigated a differentiation protocol enabling the generation and selection of human iPSC-derived MSCs exhibiting relevant surface marker expression profiles (CD105 and CD73) and functional characteristics. We generated such iPSC-MSCs from fibroblasts and bone marrow MSCs utilizing two different reprogramming constructs. All such iPSC-MSCs exhibited the characteristics of normal bone marrow-derived (BM) MSCs. In direct comparison to BM-MSCs our iPSC-MSCs exhibited a similar surface marker expression profile but shorter doubling times without reaching senescence within 20 passages. Considering functional capabilities, iPSC-MSCs provided supportive feeder layer for CD34(+) hematopoietic stem cells' self-renewal and colony forming capacities. Furthermore, iPSC-MSCs gained immunomodulatory function to suppress CD4(+) cell proliferation, reduce proinflammatory cytokines in mixed lymphocyte reaction, and increase regulatory CD4(+)/CD69(+)/CD25(+) T-lymphocyte population. In conclusion, we generated fully functional MSCs from various iPSC lines irrespective of their starting cell source or reprogramming factor composition and we suggest that such iPSC-MSCs allow repetitive cell applications for advanced therapeutic approaches.

Mousavi Niri, N., et al. (2009). "Mesenchymal stem cells do not suppress lymphoblastic leukemic cell line proliferation." Iran J Immunol **6**(4): 186-194.

BACKGROUND: Several studies have demonstrated the immunosuppresive effects of mesenchymal stem cells (MSCs) in allogeneic or mitogenic interactions. Cell-cell contact inhibition and secretion of suppressive soluble factors have been suggested in this regard. OBJECTIVE: To investigate if adipose derived MSCs could inhibit Jurkat lymphoblastic leukemia T cell proliferation during coculture. METHODS: Adherent cells with the ability of cellular growth were isolated from normal adipose tissues. Initial characterization of growing cells by flow cytometry suggested their mesenchymal stem cell characteristics. Cells were maintained in culture and used during third to fifth culture passages. Jurkat or allogeneic peripheral blood mononuclear cells (PBMCs) were labeled with carboxy fluorescein diacetate succinimidyl ester and cocultured with increasing doses of MSCs or MSC culture supernatant. Proliferation of PBMCs or Jurkat cells under these conditions was assessed by flow cytometry after 2 and 3 days of coculture, respectively. RESULTS: Results showed the expression of CD105, CD166 and CD44, and the absence of CD45, CD34 and CD14 on the surface of MSC like cells. Moreover, initial differentiation studies showed the potential of cell differentiation into hepatocytes. Comparison of Jurkat cell proliferation in the presence and absence of MSCs showed no significant difference, with 70% of cells displaying signs of at least one cell division. Similarly, the highest concentration of MSC culture supernatant (50% vol/vol) had no significant effect on Jurkat cell proliferation ($p>0.6$). The same MSC lots significantly suppressed the allogeneic PHA activated PBMCs under similar culture conditions. CONCLUSION: Using Jurkat cells as a model of leukemia T cells, our results indicated an uncertainty about the suppressive effect of MSCs and their inhibitory metabolites on tumor or leukemia cell proliferation. Additional systematic studies with MSCs of different sources are needed to fully characterize the immunological properties of MSCs before planning clinical applications.

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.

Narbona-Carceles, J., et al. (2014). "Bone marrow mesenchymal stem cell aspirates from alternative sources: is the knee as good as the iliac crest?" Injury **45 Suppl 4**: S42-47.

INTRODUCTION: The most common method to obtain human mesenchymal stem cells (MSCs) is bone marrow aspiration from the iliac crest, but MSCs have also been isolated from different bones. The main purpose of this study was to compare bone marrow MSCs aspirated from the metaphysis of the distal femur and the proximal tibia with those obtained from the iliac crest, and to determine whether these locations represent potential alternative sources of MSCs for research and clinical application. MATERIALS AND METHODS: Bone marrow was aspirated from the iliac crest and the metaphysis of the distal femur and the proximal tibia during total knee arthroplasty in 20 patients. The aspirates were centrifuged by density gradient, then mononucleated cell (MNC) concentration in the different aspirates was determined using a Coulter counter. MSCs were isolated, cultivated and characterised by their immunophenotype and by their in vitro potential for differentiation into osteoblasts, chondroblasts and adipocytes in specific media. Expansion and cell viability were quantified using trypan blue staining and cell counting with a haemocytometer (Neubauer chamber). The three sources were compared in terms of MNC concentration, viability of the cultures and presence of MSC using the Wilcoxon test. RESULTS: MNC concentration was significantly higher in the iliac crest (10.05 Millions/ml) compared with the femur (0.67 Millions/ml) and tibia (1.7 Millions/ml). Culture success rates were 90%, 71% and 47% for MSCs from the iliac crest, femur and tibia, respectively. Flow cytometry analysis showed the presence of CD90+, CD105+, CD73+, VEGF+, CD71+, HLA-DR-, CD45-, CD34-, CD19-, and CD14- cells. The immunophenotype pattern of MSCs was similar for the three locations. Trilineage differentiation was achieved with all samples. CONCLUSIONS: MSCs can be found in bone marrow from the metaphysis of both the distal femur and the proximal tibia. The phenotype and differentiation potential of these cells are similar to those of bone marrow MSCs from the iliac crest. Bone marrow aspiration from these locations is a relatively easy and safe alternative to that from the iliac crest for obtaining MSCs. Further study is required to assess whether the concentrations of MSCs obtained from these sources are sufficient for one-step therapeutic purposes.

Nazari-Shafti, T. Z., et al. (2011). "Mesenchymal stem cell derived hematopoietic cells are permissive to HIV-1 infection." Retrovirology **8**(1): 3.

BACKGROUND: Tissue resident mesenchymal stem cells (MSCs) are multipotent, self-renewing cells known for their differentiation potential into cells of mesenchymal lineage. The ability of single cell clones isolated from adipose tissue resident MSCs (ASCs) to differentiate into cells of hematopoietic lineage has been previously demonstrated. In the present study, we investigated if the hematopoietic differentiated (HD) cells derived from ASCs could productively be infected with HIV-1. RESULTS: HD cells were generated by differentiating clonally expanded cultures of adherent subsets of ASCs (CD90+, CD105+, CD45-, and CD34-). Transcriptome analysis revealed that HD cells acquire a number of elements that increase their susceptibility for HIV-1 infection, including HIV-1 receptor/co-

receptor and other key cellular cofactors. HIV-1 infected HD cells (HD-HIV) showed elevated p24 protein and gag and tat gene expression, implying a high and productive infection. HD-HIV cells showed decreased CD4, but significant increase in the expression of CCR5, CXCR4, Nef-associated factor HCK, and Vpu-associated factor BTRC. HIV-1 restricting factors like APOBEC3F and TRIM5 also showed up regulation. HIV-1 infection increased apoptosis and cell cycle regulatory genes in HD cells. Although undifferentiated ASCs failed to show productive infection, HIV-1 exposure increased the expression of several hematopoietic lineage associated genes such as c-Kit, MMD2, and IL-10. CONCLUSIONS: Considering the presence of profuse amounts of ASCs in different tissues, these findings suggest the possible role that could be played by HD cells derived from ASCs in HIV-1 infection. The undifferentiated ASCs were non-permissive to HIV-1 infection; however, HIV-1 exposure increased the expression of some hematopoietic lineage related genes. The findings relate the importance of ASCs in HIV-1 research and facilitate the understanding of the disease process and management strategies.

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.

Oedayrajsingh-Varma, M. J., et al. (2006). "Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure." Cytotherapy **8**(2): 166-177.

BACKGROUND: Adipose tissue contains a stromal vascular fraction that can be easily isolated and provides a rich source of adipose tissue-derived mesenchymal stem cells (ASC). These ASC are a potential source of cells for tissue engineering. We studied whether the yield and growth characteristics of ASC were affected by the type of surgical procedure used for adipose tissue harvesting, i.e. resection, tumescent liposuction and ultrasound-assisted liposuction. METHODS: Frequencies of ASC in the stromal vascular fraction were assessed in limiting dilution assays. The phenotypical marker profile of ASC was determined, using flow cytometry, and growth kinetics were investigated in culture. ASC were cultured under chondrogenic and osteogenic conditions to confirm their differentiation potential. RESULTS: The number of viable cells in the stromal vascular fraction was affected by

neither the type of surgical procedure nor the anatomical site of the body from where the adipose tissue was harvested. After all three surgical procedures, cultured ASC did express a CD34+ CD31- CD105+ CD166+ CD45- CD90+ ASC phenotype. However, ultrasound-assisted liposuction resulted in a lower frequency of proliferating ASC, as well as a longer population doubling time of ASC, compared with resection. ASC demonstrated chondrogenic and osteogenic differentiation potential. DISCUSSION: We conclude that yield and growth characteristics of ASC are affected by the type of surgical procedure used for adipose tissue harvesting. Resection and tumescent liposuction seem to be preferable above ultrasound-assisted liposuction for tissue-engineering purposes.

Oh, J. S., et al. (2010). "Hypoxia-preconditioned adipose tissue-derived mesenchymal stem cell increase the survival and gene expression of engineered neural stem cells in a spinal cord injury model." Neurosci Lett **472**(3): 215-219.

Hypoxic preconditioning (HP) is a novel strategy to make stem cells resistant to the ischemic environment they encounter after transplantation into injured tissue; this strategy improves survival of both the transplanted cells and the host cells at the injury site. Using both in vitro and in vivo injury models, we confirmed that HP-treated adipose tissue-derived mesenchymal stem cells (HP-AT-MSCs) increased cell survival and enhanced the expression of marker genes in DsRed-engineered neural stem cells (NSCs-DsRed). Similar to untreated AT-MSCs, HP-AT-MSCs had normal morphology and were positive for the cell surface markers CD90, CD105, and CD29, but not CD31. In three in vitro ischemic-mimicking injury models, HP-AT-MSCs significantly increased both the viability of NSCs-DsRed and the expression of DsRed and clearly reduced the number of annexin-V-positive apoptotic NSCs-DsRed and the expression of the apoptotic factor Bax. Consistent with the in vitro assay, co-transplantation of NSCs-DsRed with HP-AT-MSCs significantly improved the survival of the NSCs-DsRed, resulting in an increased expression of the DsRed reporter gene at the transplantation site in a rat spinal cord injury (SCI) model. These findings suggest that the co-transplantation of HP-AT-MSCs with engineered NSCs can improve both the cell survival and the gene expression of the engineered NSCs, indicating that this novel strategy can be used to augment the therapeutic efficacy of combined stem cell and gene therapies for SCI.

Ouchi, T., et al. (2018). "Recurrent Spindle Cell Carcinoma Shows Features of Mesenchymal Stem Cells." J Dent Res **97**(7): 779-786.

This study investigated a case of spindle cell carcinoma (SpCC) in tongue pathological lesions. The patient experienced a local recurrence and distant metastasis after surgical intervention. Although standard chemotherapy was administered, a granulomatous mass continued to develop. This aggressive growth led to survival of the tumor. Secondary debulking surgery was performed to improve the patient's quality of life at the request of the patient. Using a tissue sample derived from the secondary debulking surgery, we performed an analysis of the tumor's cell surface antigens, differentiation potential, metastatic ability, and inhibition potential by anticancer reagents. In vitro analysis revealed that the cell population grown under adherent culture conditions expressed the mesenchymal stem cell (MSC) markers CD73, CD90, and CD105. The cell line established from this SpCC contained colony-forming unit fibroblasts (CFU-Fs) and exhibited multipotent differentiation into several mesenchymal lineages, including bone, cartilage, and fat. The SpCC cells also displayed vigorous mobilization. These characteristics suggested that they had the differentiation potential of mesenchymal cells, especially MSCs, rather than that of epithelial cells. The surgical specimen analyzed in this study resisted the molecular target reagent cetuximab, which is an epidermal growth factor receptor inhibitor. This clinical insight revealed that chemotherapy-resistant SpCC cells have different characteristics compared to most other cancer cells, which are sensitive to cetuximab. Our cell death assay revealed that SpCC cell death was induced by the anticancer drug imatinib, which is known to inhibit protein tyrosine kinase activity of ABL, platelet-derived growth factor receptor alpha (PDGFRalpha), and KIT. Here, we report recurrent SpCC with characteristics of MSCs and potential for treatment with imatinib.

P, M., et al. (2011). "Adult mesenchymal stem cells and cell surface characterization - a systematic review of the literature." Open Orthop J **5**(Suppl 2): 253-260.

Human adult mesenchymal stem cells (MSCs) were first identified by Friedenstein et al. when observing a group of cells that developed into fibroblastic colony forming cells (CFU-F). Ever since, the therapeutic uses and clinical applications of these cells have increased research and interest in this field. MSCs have the potential to be used in tissue engineering, gene therapy, transplants and tissue injuries. However, identifying these cells can be a challenge. Moreover, there are no articles bringing together and summarizing the cell surface markers of MSCs in adults. The purpose of this study is to summarize all the available information about the cell surface characterization of adult human MSCs by identifying and evaluating all the published literature in this field. We have found that the most commonly reported positive markers are CD105, CD90, CD44, CD73, CD29, CD13, CD34, CD146, CD106, CD54 and CD166. The most frequently reported negative markers are CD34, CD14, CD45, CD11b, CD49d, CD106, CD10 and CD31. A number of other cell surface markers including STRO-1, SH2, SH3, SH4, HLA-A, HLA-B, HLA-C, HLA-DR, HLA-I, DP, EMA, DQ (MHC Class II), CDIO5, Oct 4, Oct 4A, Nanog, Sox-2, TERT, Stat-3, fibroblast surface antigen, smooth muscle alpha-actin, vimentin, integrin subunits alpha4, alpha5, beta1, integrins alphavbeta3 and alphavbeta5 and ICAM-1 have also been reported. Nevertheless, there is great discrepancy and inconsistency concerning the information available on the cell surface profile of adult MSCs and we suggest that further research is needed in this field to overcome the problem.

Paldino, E., et al. (2014). "Induction of dopaminergic neurons from human Wharton's jelly mesenchymal stem cell by forskolin." J Cell Physiol **229**(2): 232-244.

The purpose of this study was to investigate the Wharton's jelly mesenchymal stem cells differentiation ability toward neuronal fate. Human Wharton's jelly mesenchymal stem cells (hWJMSC) have been isolated from human umbilical cord of full-term births and characterized by flow cytometry analysis for their stem mesenchymal properties through specific surface markers expression (CD73, CD90, and CD105). hWJMSC mesodermal lineage differentiation ability and karyotype analysis were assessed. The trans-differentiation of hWJMSC into neural lineage was investigated in presence of forskolin, an agent known to increase the intracellular levels of cAMP. A molecular profile of differentiated hWJMSC was performed by microarray technology which revealed 1,532 statistically significant modulated genes respect to control cells. Most of these genes are mainly involved in functional neuronal signaling pathways and part of them are specifically required for the neuronal dopaminergic induction. The acquisition of the dopaminergic phenotype was evaluated via immunocytochemistry and Western blot analysis revealed the significant induction of Nurr1, NeuroD1, and TH proteins expression in forskolin-induced hWJMSC. Moreover, the treatment with forskolin promoted, in hWJMSC, a strong upregulation of the neurotrophin Trk receptors related to the high release of brain-derived neurotrophic factor. Taken together these findings show that hWJMSC may be represent an optimal therapeutic strategy for neurological diseases.

Pao, S. I., et al. (2017). "Effect of microgravity on the mesenchymal stem cell characteristics of limbal fibroblasts." J Chin Med Assoc **80**(9): 595-607.

BACKGROUND: Mesenchymal stem cells (MSCs) are important for regenerative medicine. Limbal fibroblasts (LFs), present in the corneal limbus, have been shown to possess MSC characteristics, and can differentiate into other cell types. The current study sought to investigate the effect of microgravity on the proliferation and differentiation of LFs, and identify culture conditions to obtain a high proportion of LFs possessing MSC characteristics. METHODS: A rotary cell culture system was used to generate microgravity. Cellular proliferation and MSC marker (CD14, CD45, CD90, CD105, and SSEA4) expression were evaluated by WST-1 test and flow cytometry, respectively. Differentiation of LFs into adipocytes, osteocytes, and chondrocytes was examined. The effects of LF-conditioned medium on limbal stem cell differentiation were assessed. RESULTS: The cellular proliferation rates under microgravity were significantly lower than those under normal gravity (0.44 vs. 0.18 at 24 h, and 0.70 vs. 0.44 at 48 h, both P </= 0.004). Higher proportions of cells expressed CD90 (95.33% vs 81.69%), CD105 (95.32% vs 87.96%), and SSEA4 (68.26% vs 26.13%) under microgravity than under normal gravity. The differentiation potential of LFs was more

prominent under microgravity. The LF-conditioned medium attenuated the differentiation of limbal corneal epithelial stem cells. CONCLUSION: Under microgravity, LFs showed a higher proportion of MSC characteristics and were easily induced into different linage cells. Culture in a microgravity environment may allow harvesting a greater number of MSC-like LFs for stem cell therapy in ocular surface reconstruction.

Park, S., et al. (2013). "Comparison of human first and third trimester placental mesenchymal stem cell." Cell Biol Int **37**(3): 242-249.

Placenta mesenchymal stem cells (PMSCs) have the characteristic features of stem cells including renewability in vitro, surface expression, differentiation potency and ability to adhere to the culture surface. PMSCs expressed genes are normally found in the embryonic tissues before the onset of gastrulation, indicating multipotency. However, the stemness can depend on the stages of the placenta from which the cells were isolated. PMSCs were isolated from two different stages of placenta for comparison, that is the first and third trimesters. Both sets had very similar patterns of surface expression as CD44, CD73, CD90 and CD105, and of self renewability in vitro. Expressions of pluripotency-coupled genes were also confirmed in both sets of cells; however, there was a significant difference in the expression levels: fPMSC (mesenchymal stem cells isolated from the first trimester human placenta) being 2-11-fold higher than tPMSC (mesenchymal stem cells isolated from the third trimester human placenta). Possibly due to the difference in the expression levels of the pluripotency-related genes, induction of genes specific to the ectodermal tissues were more prominent in fPMSC than tPMSC after induced differentiation.

Potdar, P. and R. Subedi (2011). "Defining Molecular Phenotypes of Mesenchymal and hematopoietic Stem Cells derived from Peripheral blood of Acute Lymphocytic Leukemia patients for regenerative stem cell therapy." J Stem Cells Regen Med **7**(1): 29-40.

Acute Lymphocytic Leukemia (ALL) is a clonal myeloid disorder affecting all age groups, characterized by accumulation of immature blast cells in bone marrow and in peripheral blood. Autologous Bone Marrow Transplantation is a present treatment for cure of ALL patients, which is very expensive, invasive process and may have possibility of transplantation of malignant stem cells to patients. In the present study, we hypothesized to isolate large number of normal Mesenchymal & Hematopoietic stem cells from peripheral blood of ALL patients, which will be further characterized for their normal phenotypes by using specific molecular stem cell markers. This is the first study, which defines the existing phenotypes of isolated MSCs and HSCs from peripheral blood of ALL patients. We have established three cell lines in which two were Mesenchymal stem cells designated as MSCALL and MSCnsALL and one was suspension cell line designated as HSCALL. The HSCALL cell line was developed from the lymphocyte like cells secreted by MSCALL cells. Our study also showed that MSCALL from peripheral blood of ALL patient secreted hematopoietic stem cells in vitro culture. We have characterized all three-cell lines by 14 specific stem cell molecular markers. It was found that both MSC cell lines expressed CD105, CD13, and CD73 with mixed expression of CD34 and CD45 at early passage whereas, HSCALL cell line expressed prominent feature of hematopoietic stem cells such as CD34 and CD45 with mild expression of CD105 and CD13. All three-cell lines expressed LIF, OCT4, NANOG, SOX2, IL6, and DAPK. These cells mildly expressed COX2 and did not express BCR-ABL. Overall it was shown that isolated MSCs and HSCs can be use as a model system to study the mechanism of leukemia at stem cell level and their use in stem cell regeneration therapy for Acute Lymphocytic Leukemia.

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.

Pouryazdanpanah, N., et al. (2018). "Use of Some Additives for Improving Mesenchymal Stem Cell Isolation Outcomes in Non-Mobilized Peripheral Blood." Arch Iran Med **21**(8): 362-367.

BACKGROUND: The mesenchymal stem cells (MSCs) of peripheral blood (PB) have been recognized as a promising source for allogeneic cell therapy. The objective of the present study was to isolate and characterize MSCs derived from non-mobilized PB, and evaluate their differentiation potential. METHODS: The buffy coat mononuclear fractions of the PB were concentrated using the Ficoll-Paque density gradient centrifugation and were grown on primary and secondary culture media, respectively. The isolated cells were characterized using a multidisciplinary approach which was based on morphology, immunophenotyping, gene expression, and multipotentiality. Flow cytometry and Reverse transcription polymerase chain reaction (RT-PCR) were used to identify the expression of different MSC markers. Finally, after culturing in osteogenic and adipogenic induction media, the isolated cells were stained by Alizarin red and Oil-Red O. RESULTS: In spite of absence of any bone marrow stimulating factor, the isolation approach in this study yielded a rather homogeneous and spindle-shaped mononuclear cell population (the yield of passage 0 was 0.65 +/- 0.15) that stained positive for CD90, CD105, and CD73, and were negative for CD45 and CD34. These cells have high proliferative capacity (confirmed by the expression of Oct-4, Nucleostemin, and Nanog genes) and were able to differentiate into lineage-specific committed cells, when exposed to the appropriate medium. CONCLUSION: Overall, it can be concluded that conventional, labour-intensive and time-consuming approaches are not necessary in isolating MSCs from PB. This relatively accessible and minimally invasive source, PB, represents a good alternative reservoir of homogeneous MSCs that could open a new era for practical exploitation in regenerative medicine.

Prados, A., et al. (2018). "Characterization of mesenchymal stem/stromal cells with lymphoid tissue organizer cell potential in tonsils from children." Eur J Immunol **48**(5): 829-843.

Lymphoid tissue organizer (LTo) cells, identified in mouse and human embryos, are thought to be precursors of stromal cells in secondary lymphoid organs. Whether LTo cells are present in human adults, however remains unknown. We obtained 15 stromal cell lines from tonsils from children who underwent tonsillectomy, and studied the antigen phenotype of these tonsil stromal cell (TSC) lines by flow cytometry and RT-PCR. Cell lines met the minimal criteria proposed by the International Society for Cellular Therapy to define human mesenchymal stem/stromal cells (MSCs): plastic-adherent capacity; expression of CD73, CD90 and CD105, lack of CD45, CD19 and HLA-DR; and capacity to differentiate into adipocytes, osteoblasts and chondrocytes. Furthermore, our TSC lines exhibited an antigen phenotype and functional characteristics very similar to those seen in murine embryo LTo cells: they expressed chemokines CCL19, CCL21 and CXCL13, cytokines TRANCE and IL-7, and adhesion molecules ICAM-1, mucosal addressin cell adhesion molecule (MadCAM)-1 and VCAM-1. The expression of LTo cell-associated markers and functions were upregulated by lymphotoxin (LT)alpha1beta2 and TNF, two cytokines involved in the development and maturation of secondary lymphoid tissues. Our results show that TSCs are tonsil MSCs that differentiate into LTo-

like cells in response to the effects of these cytokines.

Rahman, M. M., et al. (2014). "CD13 promotes mesenchymal stem cell-mediated regeneration of ischemic muscle." Front Physiol **4**: 402.

Mesenchymal stem cells (MSCs) are multipotent, tissue-resident cells that can facilitate tissue regeneration and thus, show great promise as potential therapeutic agents. Functional MSCs have been isolated and characterized from a wide array of adult tissues and are universally identified by the shared expression of a core panel of MSCs markers. One of these markers is the multifunctional cell surface peptidase CD13 that has been shown to be expressed on human and murine MSCs from many tissues. To investigate whether this universal expression indicates a functional role for CD13 in MSC biology we isolated, expanded and characterized MSCs from bone marrow of wild type (WT) and CD13(KO) mice. Characterization of these cells demonstrated that both WT and CD13(KO) MSCs expressed the full complement of MSC markers (CD29, CD44, CD49e, CD105, Sca1), showed comparable proliferation rates and were capable of differentiating toward the adipogenic and osteogenic lineages. However, MSCs lacking CD13 were unable to differentiate into vascular cells, consistent with our previous characterization of CD13 as an angiogenic regulator. Compared to WT MSCs, adhesion and migration on various extracellular matrices of CD13(KO) MSCs were significantly impaired, which correlated with decreased phospho-FAK levels and cytoskeletal alterations. Crosslinking human MSCs with activating CD13 antibodies increased cell adhesion to endothelial monolayers and induced FAK activation in a time dependent manner. In agreement with these in vitro data, intramuscular injection of CD13(KO) MSCs in a model of severe ischemic limb injury resulted in significantly poorer perfusion, decreased ambulation, increased necrosis and impaired vascularization compared to those receiving WT MSCs. This study suggests that CD13 regulates FAK activation to promote MSC adhesion and migration, thus, contributing to MSC-mediated tissue repair. CD13 may present a viable target to enhance the efficacy of mesenchymal stem cell therapies.

Ranera, B., et al. (2011). "Immunophenotype and gene expression profiles of cell surface markers of mesenchymal stem cells derived from equine bone marrow and adipose tissue." Vet Immunol Immunopathol **144**(1-2): 147-154.

Bone marrow and adipose tissue are the two main sources of mesenchymal stem cell (MSC). The aim of this work was to analyse the immunophenotype of 7 surface markers and the expression of a panel of 13 genes coding for cell surface markers in equine bone marrow and adipose tissue-derived MSCs obtained from 9 horses at third passage. The tri-lineage differentiation was confirmed by specific staining. Equine MSCs from both sources were positive for the MSC markers CD29 and CD90, while were negative for CD44, CD73, CD105, CD45 and CD34. The gene expression of these molecules was also evaluated by reverse transcriptase real-time quantitative PCR along with the expression of 5 other MSC markers. Both populations of cells expressed CD13, CD29, CD44, CD49d, CD73, CD90, CD105, CD106, CD146 and CD166 transcripts. Significant differences in gene expression levels between BM- and AT-MSCs were observed for CD44, CD90, CD29 and CD34. Both cell types were negative for CD45 and CD31. The surface antigens tested revealed a similar phenotypic profile between horse and human MSCs, although specific differences in some surface antigens were noticed.

Rebolj, K., et al. (2018). "Hematopoietic stem cell and mesenchymal stem cell population size in bone marrow samples depends on patient's age and harvesting technique." Cytotechnology.

Mesenchymal stem cells (MSCs) are heterogeneous population of cells with great potential for regenerative medicine. MSCs are relatively easy to expand in a cell culture, however determination of their concentration in harvested tissue is more complex and is not implemented as routine procedure. To identify MSCs collected from bone marrow we have used two combinations of cell markers (CD45(-)/CD73(+)/CD90(+)/CD105(+) and CD45(-)/CD271(+)) and fibroblast colony-forming unit (CFU-F) assay. Further, in donors of various ages, mesenchymal stem cell concentration was compared with the result of CFU-F assay and with hematopoietic stem cell concentration, determined by a standardized flow cytometric assay. A positive correlation of MSC populations to the CFU-F

numbers is observed, the population of the CD45(-)/CD271(+) cells correlates better with CFU-F numbers than the population of the CD45(-)/CD73(+)/CD90(+)/CD105(+) cells. The relationship between the hematopoietic CD45(dim)/CD34(+) cell concentration and mesenchymal CFU-Fs or CD45(-)/CD271(+) cells shows a positive linear regression. An age-related quantitative reduction of hematopoietic CD45(dim)/CD34(+), mesenchymal CD45(-)/CD73(+)/CD90(+)/CD105(+) and CD45(-)/CD271(+) stem cells, and CFU-F numbers were noted. Additionally, statistically significant higher CFU-F numbers were observed when bone marrow samples were harvested from three different sites from the anterior iliac crest instead of harvesting the same sample amount only from one site.

Reppel, L., et al. (2015). "Chondrogenic induction of mesenchymal stromal/stem cells from Wharton's jelly embedded in alginate hydrogel and without added growth factor: an alternative stem cell source for cartilage tissue engineering." Stem Cell Res Ther **6**: 260.

BACKGROUND: Due to their intrinsic properties, stem cells are promising tools for new developments in tissue engineering and particularly for cartilage tissue regeneration. Although mesenchymal stromal/stem cells from bone marrow (BM-MSC) have long been the most used stem cell source in cartilage tissue engineering, they have certain limits. Thanks to their properties such as low immunogenicity and particularly chondrogenic differentiation potential, mesenchymal stromal/stem cells from Wharton's jelly (WJ-MSC) promise to be an interesting source of MSC for cartilage tissue engineering. METHODS: In this study, we propose to evaluate chondrogenic potential of WJ-MSC embedded in alginate/hyaluronic acid hydrogel over 28 days. Hydrogels were constructed by the original spraying method. Our main objective was to evaluate chondrogenic differentiation of WJ-MSC on three-dimensional scaffolds, without adding growth factors, at transcript and protein levels. We compared the results to those obtained from standard BM-MSC. RESULTS: After 3 days of culture, WJ-MSC seemed to be adapted to their new three-dimensional environment without any detectable damage. From day 14 and up to 28 days, the proportion of WJ-MSC CD73(+), CD90(+), CD105(+) and CD166(+) decreased significantly compared to monolayer marker expression. Moreover, WJ-MSC and BM-MSC showed different phenotype profiles. After 28 days of scaffold culture, our results showed strong upregulation of cartilage-specific transcript expression. WJ-MSC exhibited greater type II collagen synthesis than BM-MSC at both transcript and protein levels. Furthermore, our work highlighted a relevant result showing that WJ-MSC expressed Runx2 and type X collagen at lower levels than BM-MSC. CONCLUSIONS: Once seeded in the hydrogel scaffold, WJ-MSC and BM-MSC have different profiles of chondrogenic differentiation at both the phenotypic level and matrix synthesis. After 4 weeks, WJ-MSC, embedded in a three-dimensional environment, were able to adapt to their environment and express specific cartilage-related genes and matrix proteins. Today, WJ-MSC represent a real alternative source of stem cells for cartilage tissue engineering.

Rider, D. A., et al. (2007). "Selection using the alpha-1 integrin (CD49a) enhances the multipotentiality of the mesenchymal stem cell population from heterogeneous bone marrow stromal cells." J Mol Histol **38**(5): 449-458.

Bone marrow-derived mesenchymal stem cells consist of a developmentally heterogeneous population of cells obtained from colony forming progenitors. As these colonies express the alpha-1 integrin (CD49a), here we single-cell FACS sorted CD49a+ cells from bone marrow in order to create clones and then compared their colony forming efficiency and multilineage differentiation capacity to the unsorted cells. Following selection, 40% of the sorted CD49a+ cells formed colonies, whereas parental cells failed to form colonies following limited dilution plating at 1 cell/well. Following ex vivo expansion, clones shared a similar morphology to the parental cell line, and also demonstrated enhanced proliferation. Further analysis by flow cytometry using a panel of multilineage markers demonstrated that the CD49a+ clones had enhanced expression of CD90 and CD105 compared to unsorted cells. Culturing cells in adipogenic, osteogenic or chondrogenic medium for 7, 10 and 15 days respectively and then analysing them by quantitative PCR demonstrated that CD49a+ clones readily underwent multlineage differentiation into fat, bone and cartilage

compared to unsorted cells. These results thus support the use of CD49a selection for the enrichment of mesenchymal stem cells, and describes a strategy for selecting the most multipotential cells from a heterogeneous pool of bone marrow mononuclear stem cells.

Riekstina, U., et al. (2008). "Characterization of human skin-derived mesenchymal stem cell proliferation rate in different growth conditions." Cytotechnology **58**(3): 153-162.

This study investigated conditions for optimal in vitro propagation of human skin-derived mesenchymal stem cells (S-MSC). Forty primary skin-derived precursor cell (SKP) cultures were established from both male and female donors (age 29-65 years) and eight of them were randomly selected for in-depth characterization. Effects of basic fibroblast growth factor (FGF-2), epidermal growth factor (EGF), leukemia inhibiting factor (LIF) and dibutyryl-cyclic adenosine monophosphate (db-cAMP) on S-MSC proliferation were investigated. Primary SKP cultures were >95% homogenous for CD90, CD73, and CD105 marker expression enabling to classify these cells as S-MSC. FGF-2 dose-dependent stimulation was observed in low serum medium only, whereas EGF neither stimulated S-MSC proliferation nor potentates the effect of FGF-2. Pronounced donor to donor differences among S-MSC cultures were observed in 3-day proliferation assay. This study demonstrates that homogenous S-MSC populations can be reproducibly isolated from individual donors of different age. Optimal cell culture conditions for in vitro propagation of S-MSC are B27 supplemented or low serum media with FGF-2 (4 ng/ml). EGF and LIF as well as db-cAMP are dispensable for S-MSC proliferation.

Rodriguez-Lozano, F. J., et al. (2014). "Effects of composite films of silk fibroin and graphene oxide on the proliferation, cell viability and mesenchymal phenotype of periodontal ligament stem cells." J Mater Sci Mater Med **25**(12): 2731-2741.

In regenerative dentistry, stem cell-based therapy often requires a scaffold to deliver cells and/or growth factors to the injured site. Graphene oxide (GO) and silk fibroin (SF) are promising biomaterials for tissue engineering as they are both non toxic and promote cell proliferation. On the other hand, periodontal ligament stem cells (PDLSCs) are mesenchymal stem cells readily accessible with a promising use in cell therapy. The purpose of this study was to investigate the effects of composite films of GO, SF and GO combined with fibroin in the mesenchymal phenotype, viability, adhesion and proliferation rate of PDLSCs. PDLSCs obtained from healthy extracted teeth were cultured on GO, SF or combination of GO and SF films up to 10 days. Adhesion level of PDSCs on the different biomaterials were evaluated after 12 h of culture, whereas proliferation rate of cells was assessed using the MTT assay. Level of apoptosis was determined using Annexin-V and 7-AAD and mesenchymal markers expression of PDLSCs were analyzed by flow cytometry. At day 7 of culture, MTT experiments showed a high rate of proliferation of PDLSCs growing on GO films compared to the other tested biomaterials, although it was slightly lower than in plastic (control). However PDLSCs growing in fibroin or GO plus fibroin films showed a discrete proliferation. Importantly, at day 10 of culture it was observed a significant increase in PDLSCs proliferation rate in GO films compared to plastic ($P < 0.05$), as well as in GO plus fibroin compared to fibroin alone ($P < 0.001$). Flow cytometry analysis showed that culture of PDLSCs in fibroin, GO or GO plus fibroin films did not significantly alter the level of expression of the mesenchymal markers CD73, CD90 or CD105 up to 168 h, being the cell viability in GO even better than obtained in plastic. Our findings suggest that the combination of human dental stem cells/fibroin/GO based-bioengineered constructs have strong potential for their therapeutic use in regenerative dentistry.

Sabatini, F., et al. (2005). "Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities." Lab Invest **85**(8): 962-971.

Mesenchymal stem cells (MSCs) are multipotent cells able to differentiate along different pathways including chondrogenic, osteogenic and adipogenic lineages. MSCs with a fibroblast-like morphology have been identified in human fetal lung. However, their frequency and characterization in human adult lung have not been yet evaluated. Therefore, we analyzed the mesenchymal phenotype and differentiation ability of cultured human adult bronchial fibroblast-like cells (Br) in

comparison with those of mesenchymal cell progenitors isolated from fetal lung (ICIG7) and adult bone marrow (BM212) tissues. Surface immunophenotyping by flow cytometry revealed a similar expression pattern of antigens characteristic of marrow-derived MSCs, including CD34 (-), CD45 (-), CD90/Thy-1 (+), CD73/SH3, SH4 (+), CD105/SH2 (+) and CD166/ALCAM (+) in Br, ICIG7 and BM212 cells. There was one exception, STRO-1 antigen, which was only weakly expressed in Br cells. Analysis of cytoskeleton and matrix composition by immunostaining showed that lung and marrow-derived cells homogeneously expressed vimentin and nestin proteins in intermediate filaments while they were all devoid of epithelial cytokeratins. Additionally, alpha-smooth muscle actin was also present in microfilaments of a low number of cells. All cell types predominantly produced collagen and fibronectin extracellular matrix as evidenced by staining with the monoclonal antibodies to collagen prolyl 4-hydroxylase and fibronectin isoforms containing the extradomain (ED)-A together with ED-B in ICIG7 cells. Br cells similarly to fetal lung and marrow fibroblasts were able to differentiate along the three adipogenic, osteogenic and chondrogenic mesenchymal pathways when cultured under appropriate inducible conditions. Altogether, these data indicate that MSCs are present in human adult lung. They may be actively involved in lung tissue repair under physiological and pathological circumstances.

Sadikovic, B., et al. (2011). "Immunohistochemical expression and cluster analysis of mesenchymal and neural stem cell-associated proteins in pediatric soft tissue sarcomas." Pediatr Dev Pathol **14**(4): 259-272.

Pediatric undifferentiated soft tissue sarcomas (USTSs) are a group of malignancies composed predominantly of primitive round cell sarcomas, the histogenesis of which is uncertain. Thus, diagnosis and therapy remain a challenge. The aims of the current study were to determine whether differential expression of stem cell-associated proteins could be used to aid in determining the histogenesis of pediatric USTSs and to determine whether pediatric USTSs expressed a unique panel of stem cell-associated proteins to aid diagnosis. Tumors included 28 Ewing sarcoma/primitive neuroectodermal tumors (ESs), 22 embryonal rhabdomyosarcomas (ERMSs), 8 alveolar rhabdomyosarcomas (ARMSs), 5 synovial sarcomas (SSs), 5 malignant peripheral nerve sheath tumors (MPNSTs), and 13 USTSs. Stem cell antibodies included 3 mesenchymal stem cell markers (CD44, CD105, and CD166) and 5 neural stem cell markers (CD15, CD29, CD56, CD133, and nestin). Sections were scored followed by statistical analysis, clustering analysis, and visualizations using Partek Genomic Suite Software. The Euclidean clustering divided the tumors into 2 major groups. ESs and USTSs formed the majority of the 1st group, whereas ERMSs, ARMSs, MPNSTs, and SSs formed the 2nd group. Reduced expression of CD56 was strongly associated with the ES/USTS cluster ($P < 0.0001$). ESs and USTSs were further separated by CD166 staining, wherein increased expression was associated with ES ($P < 0.0001$). The 2nd group included the majority of other sarcomas, with no consistent separation between subtypes. The current study demonstrates the usefulness of applying stem cell markers to pediatric sarcomas and indicates that USTSs and ESs are closely related and may share a common histogenesis.

Sammons, J., et al. (2004). "The role of BMP-6, IL-6, and BMP-4 in mesenchymal stem cell-dependent bone development: effects on osteoblastic differentiation induced by parathyroid hormone and vitamin D(3)." Stem Cells Dev **13**(3): 273-280.

Human bone marrow-derived mesenchymal stem cells (MSCs) represent an ideal source for cell therapy for inherited and degenerative diseases, bone and cartilage repair, and as target for gene therapy. The role of the combination of human parathyroid hormone (PTH) and vitamin D(3) in bone formation and mineralization has been established in several osteoblast cell culture studies. The aim of the present study was to evaluate the role of this hormonal combination alone and in the presence of bone morphogenetic protein-4 (BMP-4) or-6 (BMP-6) in inducing osteogenic differentiation of human MSC. Human MSC derived from adult normal bone marrow that are positive for CD29, CD44, CD105, and CD166 and negative for CD14, CD34, and CD45, were treated with the PTH and 1,25-dihydroxyvitamin D(3) in the presence and absence of recombinant human BMP-4 or BMP6. PTH and vitamin D(3) induced high levels of expression of two key markers of bone formation: osteocalcin and alkaline

phosphatase by MSCs. BMP-6 but not BMP-4 increased osteocalcin expression induced by PTH and vitamin D(3). Both BMPs enhanced calcium formation in MSC cultures and this response was potentiated by PTH and vitamin D(3). The present results revealed a novel potent effect of PTH and vitamin D(3) plus BMPs in inducing bone development by human MSCs. These results may facilitate therapeutic utility of MSCs for bone disease and help clarify mechanisms involved in stem cell-mediated bone development.

Sarang, S. and C. Viswanathan (2016). "Umbilical Cord Derived Mesenchymal Stem Cells Useful in Insulin Production - Another Opportunity in Cell Therapy." Int J Stem Cells **9**(1): 60-69.

BACKGROUND AND OBJECTIVES: Type 1 Diabetes Mellitus (T1DM) is an autoimmune disorder resulting out of T cell mediated destruction of pancreatic beta cells. Immunomodulatory properties of mesenchymal stem cells may help to regenerate beta cells and/or prevent further destruction of remnant, unaffected beta cells in diabetes. We have assessed the ability of umbilical cord derived MSCs (UCMSCs) to differentiate into functional islet cells in vitro. METHODS AND RESULTS: We have isolated UCMSCs and allowed sequential exposure of various inducing agents and growth factors. We characterized these cells for confirmation of the presence of islet cell markers and their functionality. The spindle shaped undifferentiated UCMSCs, change their morphology to become triangular in shape. These cells then come together to form the islet like structures which then grow in size and mature over time. These cells express pancreatic and duodenal homeobox -1 (PDX-1), neurogenin 3 (Ngn-3), glucose transporter 2 (Glut 2) and other pancreatic cell markers like glucagon, somatostatin and pancreatic polypeptide and lose expression of MSC markers like CD73 and CD105. They were functionally active as demonstrated by release of physiological insulin and C-peptide in response to elevated glucose concentrations. CONCLUSIONS: Pancreatic islet like cells with desired functionality can thus be obtained in reasonable numbers from undifferentiated UCMSCs in vitro. This could help in establishing a "very definitive source" of islet like cells for cell therapy. UCMSCs could thus be a game changer in treatment of diabetes.

Savickiene, J., et al. (2016). "Senescence-Associated Molecular and Epigenetic Alterations in Mesenchymal Stem Cell Cultures from Amniotic Fluid of Normal and Fetus-Affected Pregnancy." Stem Cells Int **2016**: 2019498.

Human amniotic-fluid-derived mesenchymal stem cells (AF-MSCs) are interesting for their multilineage differentiation potential and wide range of therapeutic applications due to the ease of culture expansion. However, MSCs undergo replicative senescence. So far, the molecular mechanisms that underlie fetal diseases and cell senescence are still poorly understood. Here, we analyzed senescence-associated morphologic, molecular, and epigenetic characteristics during propagation of MSCs derived from AF of normal and fetus-affected pregnancy. AF-MSCs cultures from both cell sources displayed quite similar morphology and expression of specific cell surface (CD44, CD90, and CD105) and stemness (Oct4, Nanog, Sox2, and Rex1) markers but had interindividual variability in proliferation capability and time to reach senescence. Within passages 4 and 8, senescent cultures exhibited typical morphological features, senescence-associated beta-galactosidase activity, increased levels of p16, and decreased levels of miR-17 and miR-21 but showed differential expression of p21, p53, and ATM dependently on the onset of cell senescence. These differences correlated with changes in the level of chromatin modifiers (DNMT1 and HDAC1) and polycomb group proteins (EZH2, SUZ12, and BMI1) paralleling with changes in the expression of repressive histone marks (H3K9me3 and H3K27me3) and stemness markers (Oct4, Nanog, Sox2, and Rex1). Therefore epigenetic factors are important for AF-MSCs senescence process that may be related with individuality of donor or a fetus malignancy status.

Savickiene, J., et al. (2017). "Histone Modifications Pattern Associated With a State of Mesenchymal Stem Cell Cultures Derived From Amniotic Fluid of Normal and Fetus-Affected Gestations." J Cell Biochem **118**(11): 3744-3755.

Human amniotic fluid (AF)-derived mesenchymal stem cells (MSCs) sharing embryonic and adult stem cells characteristics are interesting by their multipotency and the usage for regenerative medicine. However, the usefulness of these cells for revealing the fetal diseases still needs to be assessed. Here, we

have analyzed the epigenetic environment in terms of histone modifications in cultures of MSCs derived from AF of normal pregnancies and those with fetal abnormalities. The comparison of MSCs samples from AF of normal pregnancies (N) and fetus-affected (P) revealed two distinct cultures by their proliferation potential (P I and P II). Cell populations from N and P I samples had similar growth characteristics and exhibited quite similar cell surface (CD44, CD90, CD105) and stemness markers (Oct4, Nanog, Sox2, Rex1) profile that was distinct in slower growing and faster senescent P II cultures. Those differences were associated with changes in 5-Cyt DNA methylation and alterations in the expression levels of chromatin modifiers (DNMT1, HDAC1/2), activating (H4ac, H3K4me3), and repressive (H3K9me2/me3, H3K27me3) histone marks. MSCs isolated from AF with the genetic or multifactorial fetal diseases (P II samples) were enriched with repressive histone marks and H4K16ac, H3K9ac, H3K14ac modifications. This study indicates that differential epigenetic environment reflects a state of AF-MSCs dependently on their growth, phenotype, and stemness characteristics suggesting a way for better understanding of epigenetic regulatory mechanisms in AF-MSCs cultures in normal and diseased gestation conditions. J. Cell. Biochem. 118: 3744-3755, 2017. (c) 2017 Wiley Periodicals, Inc.

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.

Shan, Z., et al. (2017). "Therapeutic effect of autologous compact bone-derived mesenchymal stem cell transplantation on prion disease." J Gen Virol.

Prion diseases are fatal neurodegenerative disorders of humans and animals and no effective treatments are currently available. Allogenic transplantation of immortalized human mesenchymal stem cells (MSCs) can prolong the survival of mice infected with prions. However, autologous transplantation is an appropriate model for evaluating the effects of MSCs on prion diseases. Therefore, we isolated and purified MSCs from the femur and tibia of mice as compact bone-derived MSCs (CB-MSCs). Flow cytometric analysis showed that CB-MSCs were negative for myeloid stem cell-derived cell markers CD11b and CD45, but positive for molecules such as Sca-1, CD105 and CD90.2, which are reported to be expressed on MSCs. The ability of CB-MSCs to migrate to brain extracts from prion-infected mice was confirmed by an in vitro migration assay. Intra-hippocampus transplantation of CB-MSCs at 120 days post-inoculation marginally but significantly prolonged the survival of mice infected with the Chandler prion strain. The transplantation of CB-MSCs did not influence the accumulation of disease-specific prion protein. However, the CB-MSC transplantation enhanced microglial activation, which appeared to be polarized to the M2-type activation state. These results suggest that autologous MSC transplantation is a possible treatment for prion diseases, while the modification of microglial activation may be a therapeutic target for neurodegenerative diseases.

Shao, D., et al. (2018). "Effects of oral implants with miR122modified cell sheets on rat bone marrow mesenchymal stem cells." Mol Med Rep **17**(1): 1537-1544.

The aim of the present study was to regulate the transformation of bone marrow mesenchymal stem cells (BMMSCs) to osteoblasts to promote bone formation and osseointegration surrounding oral implants. BMMSCs were cultured using the whole bone marrow adherence method. Cell surface

markers were detected by flow cytometry, and multilineage differentiation potential was detected by osteogenic and adipogenic tests. miR122modified cell sheets were prepared by nonviral transfection and complexed with microarc titanium oxide implants to construct a genemodified tissueengineered implant, with its surface morphology observed by scanning electron microscopy (SEM). In vitro osteogenic activity of the implant was determined by alkaline phosphatase (ALP), Sirius Red, alizarin red staining, polymerase chain reaction and western blot analysis. The BMMSCs were spindle or triangularshaped. Surface markers, cluster of differentiation 29 (CD29), CD90 and CD105 were positively expressed, whereas blood cell markers CD34, CD45 and CD31 were negatively expressed. Osteogenic staining exhibited deposition of calcified nodules, while adipogenic staining demonstrated the formation of lipid droplets. miR122 modification significantly enhanced the in vitro osteogenic activity of the sheets. On day 3 of osteogenic induction, runt-related transcription factor 2, osterix, osteocalcin, collagen I, ALP and bone morphogenetic protein 2 expression levels of the experimental group were 2.0, 3.1, 4.6, 3.2, 10.5 and 4.5 times those of the blank control group, respectively. SEM imaging of the modified sheet demonstrated close adhesion and fitting between abundant cellular and extracellular matrices, and the porous surface of the implant. In vitro osteogenesis of the complex was promoted and accelerated. Thus, miR122 effectively promoted osteogenic differentiation of the BMMSC sheet. Therefore, it is feasible to construct genemodified tissueengineered implants by complexing miR122modified sheets with microarc titanium oxide implants.

Shi, S., et al. (2014). "Feasibility of lentiviralmediated sodium iodide symporter gene delivery for the efficient monitoring of bone marrowderived mesenchymal stem cell transplantation and survival." Int J Mol Med **34**(6): 1547-1554.

The aim of the present study was to explore the feasibility of lentiviral-mediated sodium iodide symporter (NIS) gene delivery for monitoring bone marrow-derived mesenchymal stem cell (BMSC) transplantation into the infarcted myocardium. For this purpose, we constructed a lentiviral vector (Lv-EF1alpha-NIS-IRES-EGFP) expressing NIS and enhanced green fluorescent protein (EGFP), and introduced it into BMSCs at different multiplicities of infection (MOI). The expression of EGFP was observed under a fluorescence microscope. Iodine uptake and the inhibition of iodine uptake by sodium perchlorate (NaClO4) in the Lv-EF1alpha-NIS-IRES-EGFPtreated BMSCs were dynamically monitored in vitro. The Lv-EF1alpha-NIS-IRES-EGFPtreated BMSCs were transplanted into the infarcted myocardium of Sprague-Dawley rats, and 99mTc99g (Tc, technetium; 99m indicates that technetium is at its excited stage; 99g indicates the atomic weight of technetium) micro-single-photon emission computed tomography (SPECT)/computed tomography (CT) imaging was performed in vivo 1 week following transplantation. The isolated BMSCs successfully differentiated into adipocytes and osteoblasts. The BMSCs were positive for the cell surface markers, CD105, CD29 and CD90, and negative for CD14, CD34 and CD45. Lv-EF1alpha-NIS-IRES-EGFP was efficiently transfected into the BMSCs. RT-qPCR and western blot analysis confirmed that the BMSCs expressed high protein and mRNA levels of NIS by day 7 following infection, and NIS expression remained at a consistent level from day 14 to 21. In the Lv-EF1alpha-NIS-IRES-EGFPtreated BMSCs, the accumulation of iodine-125 (125I) was observed in vitro and was successfully monitored by 99mTc99g micro-SPECT/CT imaging at 1 week following transplantation. These results suggest that lentiviral vectors are powerful vehicles for studying gene delivery in BMSCs. It is feasible to use lentiviral vectors to deliver an NIS gene for the non-invasive monitoring of BMSC transplantation and survival in the infarcted myocardium in vivo.

Sidney, L. E. and A. Hopkinson (2018). "Corneal keratocyte transition to mesenchymal stem cell phenotype and reversal using serum-free medium supplemented with fibroblast growth factor-2, transforming growth factor-beta3 and retinoic acid." J Tissue Eng Regen Med **12**(1): e203-e215.

Keratocytes of the corneal limbal stroma can derive populations of mesenchymal stem cells (MSC) when expanded in vitro. However, once a corneal MSC (cMSC) phenotype is achieved, regaining the keratocyte phenotype can be challenging, and there is no standardised differentiation medium. Here, we investigated the transition of keratocytes to

cMSC and compared different supplements in their ability to return cMSC to a keratocyte phenotype. Immunofluorescence and quantitative reverse transcription polymerase chain reaction demonstrated in vivo keratocyte expression of aldehyde dehydrogenase 3A1, CD34 and keratocan, but not any of the typical MSC markers (CD73, CD90, CD105). As the keratocytes were expanded in vitro, the phenotypic profile reversed and the cells expressed MSC markers but not keratocyte markers. Differentiating the cMSC back to a keratocyte phenotype using nonsupplemented, serum-free medium restored keratocyte markers but did not maintain cell viability or support corneal extracellular matrix production. Supplementing the differentiation medium with combinations of fibroblast growth factor-2, transforming growth factor-beta3 and retinoic acid maintained viability, restored expression of CD34, aldehyde dehydrogenase 3A1 and keratocan, and facilitated production of abundant extracellular matrix as shown by immunofluorescent staining for collagen-I and lumican, alongside quantitative assays for collagen and glycosaminoglycan production. However, no differentiation medium was able to downregulate the expression of MSC markers in the 21-day culture period. This study shows that the keratocyte to MSC transition can be partially reversed using serum-free media and supplementation with retinoic acid, fibroblast growth factor-2 and transforming growth factor-beta3 and can enhance this effect. This is relevant for development of corneal regenerative strategies that require the production of a keratocyte phenotype. Copyright (c) 2016 John Wiley & Sons, Ltd.

Silverio, K. G., et al. (2010). "Mesenchymal stem cell properties of periodontal ligament cells from deciduous and permanent teeth." J Periodontol **81**(8): 1207-1215.

BACKGROUND: Human postnatal stem cells have been identified in periodontal ligaments (PDLs). In this study, the in vitro biologic properties of CD105(+) enriched cell subsets from PDLs harvested from deciduous (DePDL) and permanent (PePDL) teeth are comparatively assessed. METHODS: PDL tissue was obtained from 12 teeth (six primary and six permanent) from which CD105(+) CD34(-) CD45(-) cells were isolated by magnetic cell sorting. To identify and quantitatively compare the stem cell markers, DePDL and PePDL cells were assessed for CD166 surface antigen expression by flow cytometry, real-time polymerase chain reaction, and immunostaining for Stro-1 and Oct-4, osteogenic and adipogenic differentiation, and proliferation rate by trypan blue method. RESULTS: Magnetic cell sorting isolated cell populations containing 23.87% (+/- 11.98%) and 11.68% (+/- 6.27%) of CD105(+) expressing cells from PePDL and DePDL, respectively. Flow cytometric analysis demonstrated a higher proportion of CD105(+) cells coexpressing CD166 surface antigen in PePDL, whereas immunostaining and real-time polymerase chain reaction analysis demonstrated that both cell subsets expressed Stro-1 and Oct-4. DePDL-CD105(+) subsets were more proliferative compared to PePDL subsets, and both cell populations showed multipotential capabilities to differentiate in vitro to osteoblast/cementoblast- and adipocyte-like cells. However, a higher expression of adipogenic-related genes was observed in DePDL cells, whereas PePDL-CD105(+) cell subset presented a more homogeneous osteoblast/cementoblast response. CONCLUSION: These findings demonstrate that highly purified mesenchymal progenitor cell subsets can be obtained from the PDLs of both deciduous and permanent teeth, and further indicate phenotype dissimilarities that may have an impact on their clinical applications.

Singh, S., et al. (2008). "Characterization of a mesenchymal-like stem cell population from osteophyte tissue." Stem Cells Dev **17**(2): 245-254.

Osteophytes are a distinct feature of osteoarthritis (OA). Their formation may be related to pluripotential cells in the periosteum responding to stimulus during OA. This study aimed to isolate stem cells from osteophyte tissues and to characterize their phenotype, proliferation, and differentiation potential, as well as their immunomodulatory properties. Osteophyte-derived cells were isolated from osteophyte tissue samples collected during knee replacement surgery. These cells were characterized by the expression of cell-surface antigens, differentiation potential into mesenchymal lineages, growth kinetics, and modulation of alloimmune responses. Multipotential stem cells were identified from all osteophyte samples, namely osteophyte-derived mesenchymal stem cells (oMSCs).

The surface antigen expression of oMSCs was consistent with that of MSCs; they lacked the hematopoietic and common leukocyte markers (CD34, CD45) while expressing those related to adhesion (CD29, CD166, CD44) and stem cells (CD90, CD105, CD73). The proliferation capacity of oMSCs in culture was superior to that of bone marrow-derived MSCs (bMSCs), and these cells readily differentiated into tissues of the mesenchymal lineages. oMSCs also demonstrated the ability to suppress allogeneic T cell proliferation, which was associated with the expression of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO). Our results showed that osteophyte-derived cells had similar properties to MSCs in the expression of antigen phenotype, differential potential, and suppression of alloimmune response. Furthermore, when compared to bMSCs, oMSCs maintained a higher proliferative capacity, which may offer new insights of the tissue formation and potentially an alternative source for therapeutic stem cell-based tissue regeneration.

Solodeev, I., et al. (2018). "Fas-L promotes the stem cell potency of adipose-derived mesenchymal cells." Cell Death Dis **9**(6): 695.

Fas-L is a TNF family member known to trigger cell death. It has recently become evident that Fas-L can transduce also non-apoptotic signals. Mesenchymal stem cells (MSCs) are multipotent cells that are derived from various adult tissues. Although MSCs from different tissues display common properties they also display tissue-specific characteristics. Previous works have demonstrated massive apoptosis following Fas-L treatment of bone marrow-derived MSCs both in vitro and following their administration in vivo. We therefore set to examine Fas-L-induced responses in adipose-derived stem cells (ASCs). Human ASCs were isolated from lipoaspirates and their reactivity to Fas-L treatment was examined. ASCs responded to Fas-L by simultaneous apoptosis and proliferation, which yielded a net doubling of cell quantities and a phenotypic shift, including reduced expression of CD105 and increased expression of CD73, in association with increased bone differentiation potential. Treatment of freshly isolated ASCs led to an increase in large colony forming unit fibroblasts, likely produced by early stem cell progenitor cells. Fas-L-induced apoptosis and proliferation signaling were found to be independent as caspase inhibition attenuated Fas-L-induced apoptosis without impacting proliferation, whereas inhibition of PI3K and MEK, but not of JNK, attenuated Fas-L-dependent proliferation, but not apoptosis. Thus, Fas-L signaling in ASCs leads to their expansion and phenotypic shift toward a more potent stem cell state. We speculate that these reactions ensure the survival of ASC progenitor cells encountering Fas-L-enriched environments during tissue damage and inflammation and may also enhance ASC survival following their administration in vivo.

Song, J. S., et al. (2017). "Dermatofibrosarcoma Protuberans: An Immunomarker Study of 57 Cases That Included Putative Mesenchymal Stem Cell Markers." Appl Immunohistochem Mol Morphol **25**(8): 586-591.

Dermatofibrosarcoma protuberans (DFSP) is a low-grade fibroblastic sarcoma with a superficial location that has been suggested to potentially be a type of mesenchymal stem cell tumor. We studied the expression of various immunomarkers, including putative stem cell markers, in a series of 57 DFSPs including variants, and 12 dermatofibromas (DFs). CD105, a mesenchymal stem cell marker, was weakly expressed in 24 DFSPs, whereas other stem cell markers, including CD133, ALK-1, and Oct3/4, were completely negative in all samples. The expression rates of CD105 and CD34 were significantly higher in DFSP (42% and 93%) than in DF (0% and 17%), and CD10 and D2-40 were significantly lower in DFSP (40% and 3.5%) than in DF (100% and 33%), respectively. CD99, CD117, PDGFB, and PDGFRbeta expression was comparable between the groups. CD105 mesenchymal cells were not observed in non-neoplastic dermis. In summary, we did not obtain sufficient immunohistochemical evidence to support the DFSP as a cutaneous mesenchymal stem cell tumor. CD34 alone was the most consistent marker of DFSP, irrespective of its variants. Because CD34 non-neoplastic mesenchymal cells were distributed in a location similar to that of DFSP, we suggest that DFSP might have originated from CD34 mesenchymal cells in the dermis.

Sonoda, E., et al. (2008). "A new organotypic culture of adipose tissue fragments maintains viable mature adipocytes for a long term, together with development

of immature adipocytes and mesenchymal stem cell-like cells." Endocrinology **149**(10): 4794-4798.

Adipose tissue that consists of mature and immature adipocytes is suggested to contain mesenchymal stem cells (MSCs), but a culture system for analyzing their cell types within the tissue has not been established. Here we show that three-dimensional collagen gel culture of rat sc adipose tissue fragments maintained viable mature adipocytes for a long term, producing immature adipocytes and MSC-like cells from the fragments, using immunohistochemistry, ELISA, and real time RT-PCR. Bromodeoxyuridine uptake of mature adipocytes was detected. Adiponectin and leptin, and adipocyte-specific genes of adiponectin, leptin, and PPAR-gamma were detected in culture assembly, whereas the lipogenesis factor insulin (20 mU/ml) and inflammation-related agent TNF-alpha (2 nm) increased and decreased, respectively, all of their displays. Both spindle-shaped cell types with oil red O-positive lipid droplets and those with expression of MSC markers (CD105 and CD44) developed around the fragments. The data indicate that adipose tissue-organotypic culture retains unilocular structure, proliferative ability, and some functions of mature adipocytes, generating both immature adipocytes and CD105+/CD44+ MSC-like cells. This suggests that our method will open up a new way for studying both multiple cell types within adipose tissue and the cell-based mechanisms of obesity and metabolic syndrome.

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.

Subbarao, R. B., et al. (2018). "CD105(+) Porcine Endometrial Stromal Mesenchymal Stem Cells Possess Differentiation Potential Toward Cardiomyocyte-Like Cells and Insulin-Producing beta Cell-Like Cells In Vitro." Reprod Sci: 1933719118786461.

Porcine mesenchymal stem cells (MSCs) are similar to human MSCs, hence considered a valuable model for assessing potential for cell therapy. Porcine adipose-derived MSCs (AD-MSCs) and endometrial stromal MSCs (EMSCs) displayed fibroblast-like morphology and were positive for MSC markers CD73, CD90, and CD105 and negative for hematopoietic markers CD34 and CD45. The EMSCs had similar or slightly higher growth rate compared to AD-MSCs, and similar percentage of cells of both EMSCs and AD-MSCs were at G0/G1 and G2/M phases; however, EMSCs had significantly (P < .05) higher percentage of cells at S phase of cell cycle than AD-MSCs. Transdifferentiation ability to cardiomyocyte-like cells was confirmed in differentiated cells by the expression of lineage-specific marker genes such as DES, ACTA2, cTnT, and ACTC1 by real-time quantitative polymerase chain reaction (RT-qPCR). Furthermore, cardiomyocyte-specific protein markers cTnT and ACTC1 were expressed in completely differentiated cells. Endodermal differentiation capacity of EMSCs to pancreatic beta cell-like cells was evident with the changes in morphology and the expression of beta-cell-specific marker genes such as PDX1, GLUT2, SST, NKX6.1, PAX4, and

NGN3 as analyzed by RT-qPCR. The differentiated cells secreted insulin and C-peptide upon glucose challenge and also they expressed insulin, PDX1, PAX4, NGN3, and GLUT2 at protein level as assessed by immunostaining confirming the successful differentiation to beta cell-like cells. Porcine EMSCs possess all the characteristics of MSCs and are suitable model for studying molecular mechanisms of cellular differentiation.

Subramani, B., et al. (2016). "Generation and characterization of human cardiac resident and non-resident mesenchymal stem cell." Cytotechnology **68**(5): 2061-2073.

Despite the surgical and other insertional interventions, the complete recuperation of myocardial disorders is still elusive due to the insufficiency of functioning myocardiocytes. Thus, the use of stem cells to regenerate the affected region of heart becomes a prime important. In line with this human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) have gained considerable interest due to their potential use for mesodermal cell based replacement therapy and tissue engineering. Since MSCs are harvested from various organs and anatomical locations of same organism, thus the cardiac regenerative potential of human cardiac-derived MSCs (hC-MSCs) and human umbilical cord Wharton's Jelly derived MSC (hUC-MSCs) were tested concurrently. At in vitro culture, both hUC-MSCs and hC-MSCs assumed spindle shape morphology with expression of typical MSC markers namely CD105, CD73, CD90 and CD44. Although, hUC-MSCs and hC-MSCs are identical in term of morphology and immunophenotype, yet hUC-MSCs harbored a higher cell growth as compared to the hC-MSCs. The inherent cardiac regenerative potential of both cells were further investigated with mRNA expression of ion channels. The RT-PCR results demonstrated that both MSCs were expressing a notable level of delayed rectifier-like K(+) current (I KDR) ion channel, yet the relative expression level was considerably varied between hUC-MSCs and hC-MSCs that Kv1.1(39 +/- 0.6 vs 31 +/- 0.8), Kv2.1 (6 +/- 0.2 vs 21 +/- 0.12), Kv1.5 (7.4 +/- 0.1 vs 6.8 +/- 0.06) and Kv7.3 (27 +/- 0.8 vs 13.8 +/- 0.6). Similarly, the Ca2(+)-activated K(+) current (I KCa) channel encoding gene, transient outward K(+) current (I to) and TTX-sensitive transient inward sodium current (I Na.TTX) encoding gene (Kv4.2, Kv4.3 and hNE-Na) expressions were detected in both groups as well. Despite the morphological and phenotypical similarity, the present study also confirms the existence of multiple functional ion channel currents IKDR, IKCa, Ito, and INa.TTX in undifferentiated hUC-MSCs as of hC-MSCs. Thus, the hUC-MSCs can be exploited as a potential candidate for future cardiac regeneration.

Sun, T., et al. (2008). "Pilot study on the interaction between B16 melanoma cell-line and bone-marrow derived mesenchymal stem cells." Cancer Lett **263**(1): 35-43.

Bone-marrow derived mesenchymal stem cells (BMSCs) have the potential to differentiate into osteocytes, chondrocytes, adipocytes and endothelial cells. The interaction between BMSCs and epithelial tumor cell was enhanced on proliferation. Our previous study had shown that BMSCs maybe participate in angiogenesis in melanoma in vivo. The aim of this study was to investigate the interaction between B16 melanoma cells and BMSCs in vitro, the mechanism of BMSCs participating in melanoma angiogenesis in vivo is unclear, so a co-culture system containing BMSCs and B16 melanoma cells, based on transwell indirect model, was established, and the interaction between BMSCs and B16 melanoma cells was studied in vitro. In our study, BMSCs were generated out of bone marrow from C57 mouse, isolated BMSCs were positive for the markers CD105, CD90, CD73, CD44 and CD166 and negative for endothelial markers, which acquired endothelial phenotype (including the expression of VEGFR-1, VEGFR-2, Factor VIII) after co-culture with B16 melanoma cells; at the same time, B16 melanoma cells also up-regulated the expression of VEGF-a, VEGFR-1, VEGFR-2 and Factor VIII. The proliferation rate of B16 melanoma cells and BMSCs were also found to be increased. We could show the differentiation of BMSCs into cells with phenotypic features of endothelial cells. BMSCs promoted proliferation of tumor cells and improved the microenvironment in tumor. Our study suggests that the BMSCs may play an important role in tumor angiogenesis.

Tong, C. K., et al. (2011). "Generation of mesenchymal stem cell from human umbilical cord tissue using a combination enzymatic and mechanical disassociation method." Cell Biol Int **35**(3): 221-226.

MSCs (mesenchymal stem cells) promise a great potential for regenerative medicine due to their unique properties of self-renewal, high plasticity, modulation of immune response and the flexibility for genetic modification. Therefore, the increasing demand for cellular therapy necessitates a larger-scale production of MSC; however, the technical and ethical issues had put a halt on it. To date, studies have shown that MSC could be derived from human UC (umbilical cord), which is once considered as clinical waste. We have compared the two conventional methods which are classic enzymatic digestion and explant method with our newly tailored enzymatic-mechanical disassociation method to generate UC-MSC. The generated UC-MSCs from the methods above were characterized based on their immunophenotyping, early embryonic transcription factors expression and mesodermal differentiation ability. Our results show that enzymatic-mechanical disassociation method increase the initial nucleated cell yield greatly (approximately 160-fold) and maximized the successful rate of UC-MSC generation. Enzymatic-mechanical disassociation-derived UC-MSC exhibited fibroblastic morphology and surface markers expression of CD105, CD73, CD29, CD90 and MHC class I. Furthermore, these cells constitutively express early embryonic transcription factors (Nanog, Oct-4, Sox-2 and Rex-1), as confirmed by RT-PCR, indicating their multipotency and high self-renewal capacity. They are also capable of differentiating into osteoblasts and adipocytes when given an appropriate induction. The present study demonstrates a new and efficient approach in generating MSC from UC, hence serving as ideal alternative source of mesenchymal stem cell for clinical and research use.

Touboul, C., et al. (2013). "Mesenchymal stem cells enhance ovarian cancer cell infiltration through IL6 secretion in an amniochorionic membrane based 3D model." J Transl Med **11**: 28.

BACKGROUND: The early peritoneal invasion of epithelial ovarian cancer (EOC) by tumoral aggregates presents in ascites is a major concern. The role of the microenvironment seems to be important in this process but the lack of adequate models to study cellular interactions between cancer cells and stromal cells does not allow to uncover the molecular pathways involved. Our goal was to study the interactions between ovarian cancer cells (OCC) and mesenchymal stem cells (MSC) using a 3D model. METHODS: We used millimetric pieces of amniochorionic membrane - referred to as amniotic membrane scaffold (AMS) - to create 3D peritoneal nodules mimicking EOC early invasion. We were able to measure the distribution and the depth of infiltration using confocal microsopy. We extracted MSC from the amniochorionic membrane using the markers CD34-, CD45-, CD73+, CD90+, CD105+ and CD29+ at the Fluorescence Activated Cell Sorting (FACS) analysis. We used transwell and wound healing tests to test OCC migration and invasion in vitro. RESULTS: Here we show that OCC tumors were located in regions rich in MSC (70%). The tumors infiltrated deeper within AMS in regions rich in MSC ($p<0.001$). In vitro tests revealed that higher IL6 secretion in a context of MSC-OCC co-culture could enhance migration and invasion of OCC. After IL6 receptor antagonism, OCC infiltration was significantly decreased, mostly in regions rich in MSCs, indicating that recruitment and tridimensional invasion of OCC was dependent of IL6 secretion. CONCLUSIONS: The use of tridimensional models using AMS could be a useful tool to decipher early molecular events in ovarian cancer metastasis. Cytokine inhibitors interrupting the cross-talk between OCCs and MSCs such as IL6 should be investigated as a new therapeutic approach in ovarian cancer.

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.

Tsuji, K., et al. (2017). "Effects of Different Cell-Detaching Methods on the Viability and Cell Surface Antigen Expression of Synovial Mesenchymal Stem Cells." Cell Transplant **26**(6): 1089-1102.

Flow cytometric analysis of cell surface antigens is a powerful tool for the isolation and characterization of stem cells residing in adult tissues. In contrast to the collection of hematopoietic stem cells, the process of enzymatic digestion is usually necessary to prepare mesenchymal stem cells (MSCs) suspensions, which can influence the expression of cell surface markers. In this study, we examined the effects of various cell-detaching reagents and digestion times on the expression of stem cell-related surface antigens and MSC functions. Human MSCs were detached from dishes using four different reagents: trypsin, TrypLE, collagenase, and a nonenzymatic cell dissociation reagent (C5789; Sigma-Aldrich). Following dissociation reagent incubations ranging from 5 to 120 min, cell surface markers were analyzed by flow cytometry. Trypsin and TrypLE quickly dissociated the cells within 5 min, while collagenase and C5789 required 60 min to obtain maximum cell yields. C5789 significantly decreased cell viability at 120 min. Trypsin treatment significantly reduced CD44+, CD55+, CD73+, CD105+, CD140a+, CD140b+, and CD201+ cell numbers within 30 min. Collagenase treatment reduced CD140a expression by 30 min. In contrast, TrypLE treatment did not affect the expression of any cell surface antigens tested by 30 min. Despite the significant loss of surface antigen expression after 60 min of treatment with trypsin, adverse effects of enzymatic digestion on multipotency of MSCs were limited. Overall, our data indicated that TrypLE is advantageous over other cell dissociation reagents tested for the rapid preparation of viable MSC suspensions.

Turdean, S. G., et al. (2017). "Histopathological evaluation and expression of the pluripotent mesenchymal stem cell-like markers CD105 and CD44 in the synovial membrane of patients with primary versus secondary hip osteoarthritis." J Investig Med **65**(2): 363-369.

To present the morphological changes of classic primary versus rapidly progressive and secondary hip osteoarthritis (HO) and to examine the expression of two pluripotent mesenchymal stem cell-like markers in the synovial membrane. A prospective observational study was conducted in 57 consecutive cases of radiologically confirmed HO in which total hip arthroplasty was performed. Based on the radiological and clinicopathological features, the cases were divided into three categories: classic primary HO (group A; n=16), rapidly destructive HO (group B; n=24), and HO secondary to avascular osteonecrosis of the femoral head (group C; n=17). Immunostains were performed using the markers CD44 and CD105. The cases from group A were mainly characterized by a marked perivascular inflammatory infiltrate and simple synovial hyperplasia. In group B, the papillary type of synovial hyperplasia was found and presence of chondromatosis, ossification, and ectopic follicles with germinal centers in the subsynovial layer was characteristic, whereas marked calcification and/or ossification were seen in group C. Focal expression of the CD105 and CD44 was noted in the

hyperplastic synovial cells and subsynovial layer in cases from group A, whereas synovial cells from group B were diffusely positive for both CD44 and CD105. In secondary HO, CD44 marked the inflammatory cells. Mobilization of the CD44/CD105 positive synovial cells seems to play a role in the genesis of HO. The number of the pluripotent mesenchymal stem cell-like cells derived from the hyperplastic synovial cells might be related to the severity of possible immune-mediated rapidly destructive HO.

Valenzuela, C. D., et al. (2013). "Characterization of adipose-derived mesenchymal stem cell combinations for vascularized bone engineering." Tissue Eng Part A **19**(11-12): 1373-1385.

Since bone repair and regeneration depend on vasculogenesis and osteogenesis, both of these processes are essential for successful vascularized bone engineering. Using adipose-derived stem cells (ASCs), we investigated temporal gene expression profiles, as well as bone nodule and endothelial tubule formation capacities, during osteogenic and vasculogenic ASC lineage commitment. Osteoprogenitor-enriched cell populations were found to express RUNX2, MSX2, SP7 (osterix), BGLAP (osteocalcin), SPARC (osteonectin), and SPP1 (osteopontin) in a temporally specific sequence. Irreversible commitment of ASCs to the osteogenic lineage occurred between days 6 and 9 of differentiation. Endothelioprogenitor-enriched cell populations expressed CD34, PECAM1 (CD31), ENG (CD105), FLT1 (Vascular endothelial growth factor [VEGFR1]), and KDR (VEGFR2). Capacity for microtubule formation was evident in as early as 3 days. Functional capacity was assessed in eight coculture combinations for both bone nodule and endothelial tubule formation, and the greatest expression of these end-differentiation phenotypes was observed in the combination of well-differentiated endothelial cells with less-differentiated osteoblastic cells. Taken together, our results demonstrate vascularized bone engineering utilizing ASCs is a promising enterprise, and that coculture strategies should focus on developing a more mature vascular network in combination with a less mature osteoblastic stromal cell.

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.

Vojdani, Z., et al. (2016). "The effect of amniotic membrane extract on umbilical cord blood mesenchymal stem cell expansion: is there any need to save the amniotic membrane besides the umbilical cord blood?" Iran J Basic Med Sci **19**(1): 89-96.

OBJECTIVES: Umbilical cord blood is a good source of the mesenchymal stem cells that can be banked, expanded and used in regenerative medicine. The objective of this study was to test whether amniotic membrane extract, as a rich source of growth factors such as basic-fibroblast growth factor, can promote the proliferation potential of the umbilical cord mesenchymal stem cells. MATERIALS AND METHODS: The study design was interventional. Umbilical cord mesenchymal stem cells were isolated from voluntary healthy infants from hospitals in Shiraz, Iran, cultured in the presence of basic-fibroblast growth factor and amniotic membrane extracts (from pooled - samples), and compared with control cultures. Proliferation assay was performed and duplication number and time were calculated. The expression of stem cell's specific markers and the differentiation capacity toward osteogenic and adipogenic lineages were evaluated. RESULTS: Amniotic

membrane extract led to a significant increase in the proliferation rate and duplication number and a decrease in the duplication time without any change in the cell morphology. Both amniotic membrane extract and basic-fibroblast growth factor altered the expressing of CD44 and CD105 in cell population. Treating basic-fibroblast growth factor but not the amniotic membrane extract favored the differentiation potential of the stem cells toward osteogenic lineage. CONCLUSION: The amniotic membrane extract administration accelerated cell proliferation and modified the CD marker characteristics which may be due to the induction of differentiation toward a specific lineage. Amniotic membrane extract may enhance the proliferation rate and duplication number of the stem cell through changing the duplication time.

Wang, F., et al. (2011). "Gingiva-derived mesenchymal stem cell-mediated therapeutic approach for bone tissue regeneration." Stem Cells Dev **20**(12): 2093-2102.

Up to now, the gingiva-derived mesenchymal stem cells (GMSCs) as a new postnatal stem cells have been isolated and characterized with multipotential differentiation capabilities in vitro. However, the in vivo efficacy of utilizing the GMSCs in bone regeneration remains obscure. First of all, we identified canonical MSCs in human gingival tissue, which possessed homogenous immunophenotype (CD34(-)CD45(-)CD29(+)CD105(+)CD90(+) STRO-1(+)) and had tri-lineage differentiation potential (osteoblasts, adipocytes, and chondrocytes). Next, we examined the efficacy of utilizing these stem cells in bone tissue regeneration; the enhanced green fluorescent protein-labeled GMSCs seeded on type I collagen gel were implanted into the mandibular defects as well as the critical-sized calvarial defects in Sprague Dawley rats. We first demonstrated that GMSCs could repair the mandibular wounds and calvarial defects at 2 months in rats postsurgical reconstruction. Histomorphological analysis and image of fluorescence microscope certified that new bone in the defect areas was derived from the transplanted GMSCs. Immunohistochemical analysis of green fluorescent protein, human collagen I, and osteopontin further confirmed our conclusion. The above results implied that mesenchymal stem cells derived from gingival tissue could be a novel source for stem cell-based therapy in bone reconstruction in clinical applications.

Wang, Y., et al. (2005). "Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture." Cytotherapy **7**(6): 509-519.

BACKGROUND: Human mesenchymal stem cells (hMSC) have been isolated and characterized extensively for a variety of clinical applications. Yet it is unclear how the phenomenon of hMSC plasticity can be safely and reasonably exploited for therapeutic use. METHODS: We have generated mesenchymal stem cells (MSC) from normal human BM and identified a novel cell population with a transformed phenotype. This cell population was characterized by morphologic, immunophenotypic, cytogenetic analyzes and telomerase expression. Its tumorigenicity in NOD/SCID mice was also studied. RESULTS: A subpopulation of cells in hMSC culture was noted to appear morphologically distinct from typical MSC. The cells were spherical, cuboidal to short spindle in shape, adherent and exhibited contact independent growth. Phenotypically the cells were CD133(+), CD34(-), CD45(-), CD90(low), CD105(-), VEGFR2(+). Cytogenetic analysis showed chromosome aneuploidy and translocations. These cells also showed a high level of telemerase activity compared with typical MSC. Upon transplantation into NOD/SCID mice, multiple macroscopic solid tumors formed in multiple organs or tissues. Histologically, these tumors were very poorly differentiated and showed aggressive growth with large areas of necrosis. DISCUSSION: The possible explanations for the origin of this cell population are: (1) the cells represent a transformed population of MSC that developed in culture; (2) abnormal cells existed in the donor BM at rare frequency and subsequently expanded in culture. In either case, the MSC culture may provide a suitable environment for transformed cells to expand or propagate in vitro. In summary, our data demonstrate the potential of transformed cells in hMSC culture and highlight the need for karyotyping as a release criteria for clinical use of MSC.

Wei, W., et al. (2016). "[Biological characteristics of mesenchymal stem cell and hematopoietic stem cell in

the co-culture system]." Sheng Li Xue Bao **68**(5): 691-698.

The aim of the present study was to obtain the qualified hematopoietic stem/progenitor cells (HSC/HPC) and human umbilical cord-mesenchymal stem cells (MSC) in vitro in the co-culture system. Cord blood mononuclear cells were separated from umbilical cord blood by Ficoll lymphocyte separation medium, and then CD34(+) HSC was collected by MACS immunomagnetic beads. The selected CD34(+) HSC/HPC and MSC were transferred into culture flask. IMDM culture medium with 15% AB-type cord plasma supplemented with interleukin-3 (IL-3), IL-6, thrombopoietin (TPO), stem cell factor (SCF) and FMS-like tyrosine kinase 3 ligand (Flt-3L) factors were used as the co-culture system for the amplification of HSC/HPC and MSC. The cellular growth status and proliferation on day 6 and 10 after co-culture were observed by using inverted microscope. The percentage of positive expression of CD34 in HSC/HPC, as well as the percentages of positive expressions of CD105, CD90, CD73, CD45, CD34 and HLA-DR in the 4(th) generation MSC, was tested by flow cytometry. Semisolid colony culture was used to test the HSC/HPC colony forming ability. The osteogenic, chondrogenesis and adipogenic ability of the 4(th) generation MSC were assessed. The karyotype analysis of MSC was conducted by colchicines. The results demonstrated that the HSC/HPC of co-culture group showed higher ability of amplification, CFU-GM and higher CD34(+) percentage compared with the control group. The co-cultured MSC maintained the ability to differentiate into bone cells, fat cells and chondrocytes. And the karyotype stability of MSC remained normal. These results reveal that the appropriate co-culture system for MSC and HSC is developed, and via this co-culture system we could gain both two kinds of these cells. The MSCs under the co-culture system maintain the biological characteristics. The CFU-GM ability, cell counting and the flow cytometry results of HSC/HPC under the co-culture system are conform to the criterion, showing that the biological functions of HSC/HPC are maintained.

Wittig, D., et al. (2013). "Immunohistochemical localization and characterization of putative mesenchymal stem cell markers in the retinal capillary network of rodents." Cells Tissues Organs **197**(5): 344-359.

Perivascular cells of microvascular niches are the prime candidates for being a reservoire of mesenchymal stem cell (MSC)-like cells in many tissues and organs that could serve as a potential source of cells and a target of novel cell-based therapeutic approaches. In the present study, by utilising typical markers of pericytes (neuronal-glial antigen 2, NG2, a chondroitin sulphate proteoglycan) and those of MSCs (CD146 and CD105) and primitive pluripotent cells (sex-determining region Y-box 2, Sox2), the phenotypic traits and the distribution of murine and rat retinal perivascular cells were investigated in situ. Our findings indicate that retinal microvessels of juvenile rodents are highly covered by NG2-positive branching processes of pericytic (perivascular) cells that are less prominent in mature capillary networks of the adult retina. In the adult rodent retinal vascular bed, NG2 labeling is mainly confined to membranes of the cell body resulting in a pearl-chain-like distribution along the vessels. Retinal pericytes, which were identified by their morphology and NG2 expression, simultaneously express CD146. Furthermore, CD146-positive cells located at small arteriole-to-capillary branching points appear more intensely stained than elsewhere. Evidence for a differential expression of the two markers around capillaries that would hint at a clonal heterogeneity among pericytic cells, however, is lacking. In contrast, the expression of CD105 is exclusively restricted to vascular endothelial cells and Sox2 is detected neither in perivascular nor in endothelial cells. In dissociated retinal cultures, however, simultaneous expression of NG2 and CD105 was observed. Collectively, our data indicate that vascular wall resident retinal pericytes share some phenotypic features (i.e. CD146 expression) with archetypal MSCs, which is even more striking in dissociated retinal cultures (i.e. CD105 expression). These findings might have implications for the treatment of retinal pathologies.

Xia, Z., et al. (2016). "Altered function in cartilage derived mesenchymal stem cell leads to OA-related cartilage erosion." Am J Transl Res **8**(2): 433-446.

A portion of osteoarthritis (OA) patients with total knee arthroplasty (TKA) had monocondylar destruction in medial femoral

condyle, but healthy-appearant cartilage in lateral side. However, there is limited information concerning functional differences of cartilage derived mesenchymal stem cell (CMSC) between these two locations in the same donor and its possible role in the pathogenesis of OA. Cells isolated from the degraded cartilage in medial condyle and normal cartilage in lateral side from OA patients were identified with co-expressed markers CD105 and CD166 and confirmed as CMSCs by immunophenotype. The relative percentage, proliferation activity, multi-lineage differentiation potential and miRNA expression profile of CMSCs in two groups were compared by flow cytometry, CCK-8 assay, cytochemical staining, immunohistochemistry, real-time PCR and miRNA microarray analysis. Our study suggested that the percentage (10.61+/-6.97% vs. 18.44+/-9.97%, P<0.05) and proliferation rate (P<0.01) of CD105+/CD166+ CMSCs from the degraded cartilage were significantly reduced compared with those from the normal cartilage. CMSCs from the degraded cartilage also showed stronger osteogenic (P<0.05), weaker adipogenic (P<0.01), and comparable chondrogenic potential (P>0.05) during differentiation. MiR-31-5p and miR-424-5p were down regulated in CMSCs from the degraded cartilage. In conclusion, altered function such as reduced percentage and proliferation ability, as well as changes in differentiation profile of CMSC contributed to homeostasis imbalance, leading to OA-related cartilage erosion. Furthermore, regulatory networks of multiple miRNAs may be partially responsible for the dysfunction of CMSCs.

Xiong, Z. H., et al. (2018). "Protective effect of human umbilical cord mesenchymal stem cell exosomes on preserving the morphology and angiogenesis of placenta in rats with preeclampsia." Biomed Pharmacother **105**: 1240-1247.

AIM: This study aims to investigate the effect of human umbilical cord mesenchymal stem cell exosomes (hucMSC-Ex) on placental tissue and angiogenesis in rats with preeclampsia (PE). METHOD: The expression of MSC surface markers were identified by flow cytometry. Alizarin red staining and oil red O staining were used to examine osteogenic and adipogenic differentiation of hucMSCs. Western blotting was used to determine expressions of CD63 and CD81 in hucMSC-Ex. PE rat models were established using endothelial nitric oxide synthase, eNOS)N(G)-Nitro-l-arginine Methyl Ester, which were then treated with exosome (Exo) of low dosage (L-Exo), Exo of medium dosage (M-Exo) and Exo of high dosage (H-Exo). The blood pressure at the 15d, 17d and 19d of pregnancy and 24-h urinary protein were measured. TUNEL staining and immunohistochemistry were applied to detect the cell apoptosis and micro-vascular density (MVD) in placental tissues, respectively. Enzyme-linked immunosorbent assay (ELISA) was used to examine serum levels of vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase receptor-1 (sFlt1). RESULTS: In vitro cultured hucMSCs showed expression of MSC surface markers (CD29, CD90 and CD105), and no expression of CD34 and CD45. Besides, the isolated exosomes expressed the exosome markers (CD63 and CD81). In response to the treatment of L-Exo, M-Exo and H-Exo, the blood pressure of PE rat models on the 17 d and the 19 d as well as the 24-h urinary protein were substantially decreased. Moreover, at the 21 d, PE rat models treated with L-Exo, M-Exo and H-Exo exhibited an increase in the number and quality of fetuses, placenta quality, MVD and VEGF expression, but substantial decreased cell apoptosis and expression of sFlt1. The influence of Exos was exerted in a dosage dependent manner. CONCLUSION: hucMSC-Ex, in a dose-dependent manner, can improve the morphology of placental tissue in rats with PE, by inhibiting cell apoptosis and promoting angiogenesis in placental tissue.

Xu, Y., et al. (2012). "Promising new potential for mesenchymal stem cells derived from human umbilical cord Wharton's jelly: sweat gland cell-like differentiative capacity." J Tissue Eng Regen Med **6**(8): 645-654.

Mesenchymal stem cells derived from Wharton's jelly of the human umbilical cord (hUC-MSCs) possess various advantageous properties, similar to bone marrow-derived mesenchymal stem cells (BM-MSCs), including self-renewal, extended proliferation potential and multilineage differentiation potential. In this study, we hoped to determine whether hUC-MSCs could be induced to differentiate into sweat gland cell-like cells, that would be potential in sweat glands restoration after injury. In this study, the

results of flow cytometry analysis revealed that hUC-MSCs showed the typical antigen profile of MSCs and were positive for CD29, CD44, CD90, CD105 and Oct-4; they were negative for the antigens of CD34, CEA and CK14. Remarkably, hUC-MSCs maintained proper proliferation and differentiation ability. After culture in sweat gland cell-conditioned medium (induction group 1) for 3 weeks, hUC-MSCs possessed sweat gland cell-like morphology and expressed markers of sweat gland cells (CEA, CK14 and CK19) more efficiently than those of induction group 2. In reverse-transcription PCR and western blotting analysis, it was further confirmed that induced hUC-MSCs (group 1) also expressed a higher level of sweat gland developmental genes (EDA and EDAR) than group 2. These results together provided evidence that hUC-MSCs could possess a new emerging potential to differentiate into sweat gland cell-like cells with a higher efficacy under our new induction system. Thus, hUC-MSCs could be considered a new strategy for sweat glands restoration after skin injury as well as improvement of cutaneous regeneration.

Yan, X., et al. (2012). "Mesenchymal stem cell-like cells in classic renal angiomyolipoma." Oncol Lett **4**(3): 398-402.

As a benign mesenchymal tumor, classic renal angiomyolipoma (AML) may obliterate the kidney parenchyma and cause renal hemorrhage. It has previously been reported that mesenchymal stem cells (MSCs) are involved in tumorigenesis; however, there have been no studies on stem cells with renal AML origin. In the present study, six females with classic renal AML received a partial or total nephrectomy. During surgery, tumor tissues were collected and culture expansion of adhesive fibroblastoid cells from these tissues was performed. We successfully isolated and cultured MSC-like cells from all six renal AML tumors. MSC characteristics, including morphology, immunophenotype and multidifferentiation potential were analyzed. Flow cytometry analysis revealed that these cells are highly similar to human bone marrow MSCs due to the expression of MSC-specific surface proteins, including CD29, CD44, CD73, CD90 and CD105. The stem cell-like nature of these cells is further supported by their adipogenic and osteogenic differentiation potentials when incubated in appropriate differentiation cocktails. Renal AML-derived adhesive cells possessing the characteristics of MSCs are described for the first time. They are a novel cell type which may be useful in future studies with regards to determining the role of stem cells in the formation and development of renal AML.

Yang, Y., et al. (2016). "Conditioned umbilical cord tissue provides a natural three-dimensional storage compartment as in vitro stem cell niche for human mesenchymal stroma/stem cells." Stem Cell Res Ther **7**: 28.

BACKGROUND: The use of large amounts of human multipotent mesenchymal stroma/stem cells (MSC) for cell therapies represents a desirable property in tissue engineering and banking in the field of regenerative medicine. METHODS AND RESULTS: Whereas cryo-storage of umbilical cord (UC) tissue pieces in liquid nitrogen without ingredients was associated with predominant appearance of apoptotic cells after thawing and re-culture, progressive growth of MSC was observed following use of cryo-medium. Moreover, conditioning of UC tissue pieces by initial explant culture and subsequent cryo-storage with cryo-medium accelerated a further MSC culture after thawing. These findings suggested that conditioning of UC tissue pieces provides an in vitro stem cell niche by maintenance of a 3-dimensional natural microenvironment for continuous MSC outgrowth and expansion. Indeed, culture of GFP-labeled UC tissue pieces was accompanied by increased outgrowth of GFP-labeled cells which was accelerated in conditioned UC tissue after cryo-storage. Moreover, cryopreserved conditioned UC tissue pieces in cryo-medium after thawing and explant culture could be cryopreserved again demonstrating renewed MSC outgrowth after repeated thawing with similar population doublings compared to the initial explant culture. Flow cytometry analysis of outgrowing cells revealed expression of the typical MSC markers CD73, CD90, and CD105. Furthermore, these cells demonstrated little if any senescence and cultures revealed stem cell-like characteristics by differentiation along the adipogenic, chondrogenic and osteogenic lineages. CONCLUSIONS: Expression of MSC markers was maintained for at least 10 freeze/thaw/explant culture cycles demonstrating that repeated cryopreservation

of conditioned UC tissue pieces provided a reproducible and enriched stem cell source.

Yang, Y., et al. (2015). "Human mesenchymal stroma/stem cells exchange membrane proteins and alter functionality during interaction with different tumor cell lines." Stem Cells Dev **24**(10): 1205-1222.

To analyze effects of cellular interaction between human mesenchymal stroma/stem cells (MSC) and different cancer cells, direct co-cultures were performed and revealed significant growth stimulation of the tumor populations and a variety of protein exchanges. More than 90% of MCF-7 and primary human HBCEC699 breast cancer cells as well as NIH:OVCAR-3 ovarian adenocarcinoma cells acquired CD90 proteins during MSC co-culture, respectively. Furthermore, SK-OV-3 ovarian cancer cells progressively elevated CD105 and CD90 proteins in co-culture with MSC. Primary small cell hypercalcemic ovarian carcinoma cells (SCCOHT-1) demonstrated undetectable levels of CD73 and CD105; however, both proteins were significantly increased in the presence of MSC. This co-culture-mediated protein induction was also observed at transcriptional levels and changed functionality of SCCOHT-1 cells by an acquired capability to metabolize 5'cAMP. Moreover, exchange between tumor cells and MSC worked bidirectional, as undetectable expression of epithelial cell adhesion molecule (EpCAM) in MSC significantly increased after co-culture with SK-OV-3 or NIH:OVCAR-3 cells. In addition, a small population of chimeric/hybrid cells appeared in each MSC/tumor cell co-culture by spontaneous cell fusion. Immune fluorescence demonstrated nanotube structures and exosomes between MSC and tumor cells, whereas cytochalasin-D partially abolished the intercellular protein transfer. More detailed functional analysis of FACS-separated MSC and NIH:OVCAR-3 cells after co-culture revealed the acquisition of epithelial cell-specific properties by MSC, including increased gene expression for cytokeratins and epithelial-like differentiation factors. Vice versa, a variety of transcriptional regulatory genes were down-modulated in NIH:OVCAR-3 cells after co-culture with MSC. Together, these mutual cellular interactions contributed to functional alterations in MSC and tumor cells.

Zanini, C., et al. (2011). "Differentiation of mesenchymal stem cells derived from pancreatic islets and bone marrow into islet-like cell phenotype." PLoS One **6**(12): e28175.

BACKGROUND: Regarding regenerative medicine for diabetes, accessible sources of Mesenchymal Stem Cells (MSCs) for induction of insular beta cell differentiation may be as important as mastering the differentiation process itself. METHODOLOGY/PRINCIPAL FINDINGS: In the present work, stem cells from pancreatic islets (human islet-mesenchymal stem cells, HI-MSCs) and from human bone marrow (bone marrow mesenchymal stem cells, BM-MSCs) were cultured in custom-made serum-free medium, using suitable conditions in order to induce differentiation into Islet-like Cells (ILCs). HI-MSCs and BM-MSCs were positive for the MSC markers CD105, CD73, CD90, CD29. Following this induction, HI-MSC and BM-MSC formed evident islet-like structures in the culture flasks. To investigate functional modifications after induction to ILCs, ultrastructural analysis and immunofluorescence were performed. PDX1 (pancreatic duodenal homeobox gene-1), insulin, C peptide and Glut-2 were detected in HI-ILCs whereas BM-ILCs only expressed Glut-2 and insulin. Insulin was also detected in the culture medium following glucose stimulation, confirming an initial differentiation that resulted in glucose-sensitive endocrine secretion. In order to identify proteins that were modified following differentiation from basal MSC (HI-MSCs and BM-MSCs) to their HI-ILCs and BM-ILCs counterparts, proteomic analysis was performed. Three new proteins (APOA1, ATL2 and SODM) were present in both ILC types, while other detected proteins were verified to be unique to the single individual differentiated cells lines. Hierarchical analysis underscored the limited similarities between HI-MSCs and BM-MSCs after induction of differentiation, and the persistence of relevant differences related to cells of different origin. CONCLUSIONS/SIGNIFICANCE:
Proteomic analysis highlighted differences in the MSCs according to site of origin, reflecting spontaneous differentiation and commitment. A more detailed understanding of protein assets may provide insights required to master the differentiation process of HI-MSCs to functional beta cells based only upon culture conditioning. These findings may open

new strategies for the clinical use of BM-MSCs in diabetes.

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, L., et al. (2007). "[Research on marrow mesenchymal stem cell proliferation by coculturing with Schwann cell]." Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi **21**(8): 862-866.

OBJECTIVE: To evaluate the effect of Schwann cell (SC) on the proliferation of marrow mesenchymal stem cells (MSCs) and provide evidence for application of SC in construction of the tissue engineered vessels. METHODS: SC and MSCs were harvested

from SD rats (weight 40 g). SC were verified immunohistochemically by the S-100 staining, and MSCs were verified by CD 44, CD 105, CD 34 and CD 45. The 3rd passages of both the cells were cocultured in the Transwell system and were amounted by the 3H-TDR integration technique at 1, 3, 5 and 7 days, respectively. The results were expressed by the CPM(counts per minute, CPM) values. However, MSCs on both the layers were served as the controls. The Western blot was performed to assess the expression of the vascular endothelial growth factor (VEGF), its receptor Flk-1, and the associated receptor neuropilin 1 (NRP-1) in SC, the trial cells, and the controls. RESULTS: SC had a spindle shape in the flasks, and more than 90% of SC had a positive reaction for the S-100 staining. MSCs expressed CD44 and CD105, and had a negative signal in CD 34 and CD 45. The CPM values of MSCs in the trial groups were 2,411.00+/-270.84, 3,016.17+/-241.57, 6,570.83+/-2,848.27 and 6,375.8+/-1,431.28 at 1, 3, 5 and 7 days, respectively. They were significantly higher in their values than the control group (2,142.17+/- 531.63, 2,603.33+/-389.64, 2,707.50+/-328.55, 2,389.00+/-908.01), especially at 5 days ($P<0.05$). The Western blot indicated that VEGF was expressed obviously in both the SC group and the cocultured MSCs group and was less visible in the control cells. The expressions of Flk-1 and NRP-1 in the cocultured MSCs were much stronger than in the controls. CONCLUSION: SC can significantly promote the proliferation of MSCs when they are cocultured. The peak time of the proliferation effect appeared at 5 days. This effect may be triggered by the up-regulation of VEGF in MSCs, which also leads to the upregulation of Flk-1 and NRP-1.

Zhang, R. L., et al. (2009). "[Ameliorative effect of rat bone marrow mesenchymal stem cell transplantation on infracted heart function]." Zhongguo Shi Yan Xue Ye Xue Za Zhi **17**(2): 390-394.

This study was purposed to investigate the effects of rat marrow mesenchymal stem cell (rMSC) transplantation on left ventricular (LV) function in a rat myocardial infarction model. Myocardial infarction was performed in male Lewis rats by ligating the proximal left coronary artery. Rats were randomly divided into 3 groups: sham operation group (only thoracotomy, n = 8), AMI group (DF12 injection, n = 10), rMSC group (Dil-Labeled rMSC transplantation). At 8 weeks later, the cardiac functions including left ventricular ejection fraction (LVEF), left ventricular end systolic pressure (LVESP), left ventricular end diastolic pressure (LVEDP), +dp/dtmax and -dp/dtmax were evaluated by echocardiography and cardiac catheterization. The presence and differentiation of engrafted cells were assessed. CD31 was detected by immunohistochemical staining to demonstrate neovascular formation. The results indicated that the cultured in vitro rMSC expressed CD90, CD44, CD105, CD54; did not express CD34, CD45, CD31, as compared with AMI group, rMSC group showed a significant increase of LVEF, LVESP, +dp/dtmax, -dp/dtmax and a significant decrease of LVEDP. Immunofluorescence demonstrated that some transplanted rMSCs were positive for myosin, suggesting that small number of transplanted rMSCs differentiated into cardiac-like cells. Immunostaining showed marked augmentation of capillary density in the rMSC group than that of AMI group. It is concluded that transplanted rMSCs can differentiate into cardiac-like cells and rMSC transplantation can improve LV function after myocardial infarction in rats.

Zhang, S., et al. (2013). "Neonatal desensitization supports long-term survival and functional integration of human embryonic stem cell-derived mesenchymal stem cells in rat joint cartilage without immunosuppression." Stem Cells Dev **22**(1): 90-101.

Immunological response hampers the investigation of human embryonic stem cells (hESCs) or their derivates for tissue regeneration in vivo. Immunosuppression is often used after surgery, but exhibits side effects of significant weight loss and allows only short-term observation. The purpose of this study was to investigate whether neonatal desensitization supports relative long-term survival of hESC-derived mesenchymal stem cells (hESC-MSCs) and promotes cartilage regeneration. hESC-MSCs were injected on the day of birth in rats. Six weeks after neonatal injection, a full-thickness cylindrical cartilage defect was created and transplanted with a hESC-MSC-seeded collagen bilayer scaffold (group d+s+c) or a collagen bilayer scaffold (group d+s). Rats without neonatal injection were transplanted with the hESC-MSC-seeded collagen bilayer scaffold to serve as controls (group s+c). Cartilage regeneration was evaluated by histological analysis,

immunohistochemical staining, and biomechanical test. The role of hESC-MSCs in cartilage regeneration was analyzed by CD4 immunostaining, cell death detection, and visualization of human cells in regenerated tissues. hESC-MSCs expressed CD105, CD73, CD90, CD29, and CD44, but not CD45 and CD34, and possessed trilineage differentiation potential. Group d+s+c exhibited greater International Cartilage Repair Society (ICRS) scores than group d+s or group s+c. Abundant collagen type II and improved mechanical properties were detected in group d+s+c. There were less CD4+ inflammatory cell infiltration and cell death at week 1, and hESC-MSCs were found to survive as long as 8 weeks after transplantation in group d+s+c. Our study suggests that neonatal desensitization before transplantation may be an efficient way to develop a powerful tool for preclinical study of human cell-based therapies in animal models.

Zhao, X. F., et al. (2013). "[The research of fibroblasts from human hypertrophic scar showing a mesenchymal stem cell phenotype and multilineage differentiation potentialities]." Zhonghua Zheng Xing Wai Ke Za Zhi **29**(4): 273-279.

OBJECTIVE: To explore the CD phenotypic, protein expression and pluripotent differentiation of human hypertrophic scar fibroblasts cultured in vitro, so as to study the mechanisms of scar formation. METHODS: Fibroblasts were isolated and cultured from human hypertrophic scar of 3 cases. The cells morphology was observed by inverted microscope, and the growing state of the third passage was detected by the cell counting meter of Vi-CELL. The cell surface markers CD105, CD14, CD73, CD34, CD44, CD45 and CD90 were identified by flow cytometry. The expression of CK19, Oct-4, Nanog and vimentin was detected by immunocytochemistry, and the expression of alpha-smooth muscle actin(alpha-SMA) was tested by immunofluorescence. The differentiated potential of fibroblasts of the third passage into adipogenic, osteogenic and chondrogenic lineages was assayed. RESULTS: The primary passage fibroblasts showed the shape of spindle shaped or irregular polygon with a radiated or circinate of growing arrangement. The growth curve showed the cells growth was slow on the first and second day, and quick during the third to fifth day, which reached platform stage on the sixth or seventh day. The fibroblasts highly expressed mesenchymal stem cell surface markers-CD73, CD105, CD44, CD90, but not expressed hematopoietic stem cell surface markers-CD14, CD34, CD45 by flow cytometry. And positive expression of vimentin, Oct-4 and negative expression of CK19 were detected by Immunocytochemistry. Positive expression of alpha-SMA was also detected by immunofluorescence. Multidirectional differentiation induction indicated that the third passage cells could differentiate into adipogenic, osteogenic and chondrogenic lineages. CONCLUSIONS: Human hypertrophic scar-derived fibroblasts show the biologic characteristics of mesenchymal stem cells, which may play an important role in wound healing and hypertrophic scar formation.

Zhao, Z., et al. (2005). "Establishment and properties of fetal dermis-derived mesenchymal stem cell lines: plasticity in vitro and hematopoietic protection in vivo." Bone Marrow Transplant **36**(4): 355-365.

Human mesenchymal stem cells (hMSCs) are excellent candidates for ex vivo gene transfer and cell therapy in various systems. However, hMSCs are mortal somatic cells, and thus invariably enter an irreversible growth arrest after a finite number of cell divisions in culture. It has been proposed that this is due to telomere shortening. In this study, pGRN145 plasmid containing human telomerase reverse transcriptase (hTRT) was introduced into fetal dermis-derived hMSCs. Single-cell clones positive for telomerase activity and hTRT mRNA were selected and expanded. Single-cell-derived hTRT(+) cells could be expanded rapidly in vitro and passaged up to 70 doublings without showing senescence. FACScan flow cytometer showed that hTRT(+) cells were positive for CD29, CD44, CD105, and CD166, while CD31, CD45, CD34, vWF, and HLA-DR were negative. Under suitable conditions, hTRT(+) cells have the ability of multiple lineage differentiation, including bone, fat, and nerve. Furthermore, transplantation of hTRT(+) cells could protect NOD/SCID mice from lethal irradiation. Thus, these cells may be an ideal cell source for promoting hematopoietic recovery after radiotherapy.

Zheng, Y. L., et al. (2015). "Mesenchymal Stem Cells Obtained from Synovial Fluid Mesenchymal Stem Cell-Derived Induced Pluripotent Stem Cells on a

Matrigel Coating Exhibited Enhanced Proliferation and Differentiation Potential." PLoS One **10**(12): e0144226.

Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) serve as a promising source for cell-based therapies in regenerative medicine. However, optimal methods for transforming iPSCs into MSCs and the characteristics of iPSC-MSCs obtained from different methods remain poorly understood. In this study, we developed a one-step method for obtaining iPSC-MSCs (CD146+STRO-1+ MSCs) from human synovial fluid MSC-derived induced iPSCs (SFMSC-iPSCs). CD146-STRO-1- SFMSCs were reprogrammed into iPSCs by transduction with lentivirus-mediated Sox2, Oct-3/4, klf4, and c-Myc. SFMSC-iPSCs were maintained with mTeSR1 medium in Matrigel-coated culture plates. Single dissociated cells were obtained by digesting the SFMSC-iPSCs with trypsin. The dissociated cells were then plated into Matrigel-coated culture plate with alpha minimum essential medium supplemented with 10% fetal bovine serum, 1x Glutamax, and the ROCK inhibitor Y-27632. Cells were then passaged in standard cell culture plates with alpha minimum essential medium supplemented with 10% fetal bovine serum and 1x Glutamax. After passaging in vitro, the cells showed a homogenous spindle-shape similar to their ancestor cells (SFMSCs), but with more robust proliferative activity. Flow cytometric analysis revealed typical MSC surface markers, including expression of CD73, CD90, CD105, and CD44 and lack of CD45, CD34, CD11b, CD19, and HLA-DR. However, these cells were positive for CD146 and stro-1, which the ancestor cells were not. Moreover, the cells could also be induced to differentiate in osteogenic, chondrogenic, and adipogenic lineages in vitro. The differentiation potential was improved compared with the ancestor cells in vitro. The cells were not found to exhibit oncogenicity in vivo. Therefore, the method presented herein facilitated the generation of STRO-1+CD146+ MSCs from SFMSC-iPSCs exhibiting enhanced proliferation and differentiation potential.

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