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Stem Cell

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# Stem Cell patents Research Literatures (2)

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

## Introduction

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

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

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

Mesenchymal stem/stromal cells derived from chorionic villi of human term placentae (pMSCs) produce a unique combination of molecules, which modulate important cellular functions of their target cells while concurrently suppressing their immune responses. These properties make MSCs advantageous candidates for cell-based therapy. Our first aim was to examine the effect of high levels of oxidative stress on pMSC functions. pMSCs were exposed to hydrogen peroxide (H2O2) and their ability to proliferate and adhere to an endothelial cell monolayer was determined. Oxidatively stressed pMSCs maintained their proliferation and adhesion potentials. The second aim was to measure the ability of pMSCs to prevent oxidative stress-related damage to endothelial cells. Endothelial cells were exposed to H2O2, then co-cultured with pMSCs, and the effect on endothelial cell adhesion, proliferation and migration was determined. pMSCs were able to reverse the damaging effects of oxidative stress on the proliferation and migration but not on the adhesion of endothelial cells. These data indicate that pMSCs are not only inherently resistant to oxidative stress, but also protect endothelial cell functions from oxidative stress-associated damage. Therefore, pMSCs could be used as a therapeutic tool in inflammatory diseases by reducing the effects of oxidative stress on endothelial cells.

Aguirre, A., et al. (2010). "Dynamics of bone marrow-derived endothelial progenitor cell/mesenchymal stem cell interaction in co-culture and its implications in angiogenesis." Biochem Biophys Res Commun **400**(2): 284-291.

Tissue engineering aims to regenerate tissues and organs by using cell and biomaterial-based approaches. One of the current challenges in the field is to promote proper vascularization in the implant to prevent cell death and promote host integration. Bone marrow endothelial progenitor cells (BM-EPCs) and mesenchymal stem cells (MSCs) are bone marrow resident stem cells widely

employed for proangiogenic applications. In vivo, they are likely to interact frequently both in the bone marrow and at sites of injury. In this study, the physical and biochemical interactions between BM-EPCs and MSCs in an in vitro co-culture system were investigated to further clarify their roles in vascularization. BM-EPC/MSC co-cultures established close cell-cell contacts soon after seeding and self-assembled to form elongated structures at 3days. Besides direct contact, cells also exhibited vesicle transport phenomena. When co-cultured in Matrigel, tube formation was greatly enhanced even in serum-starved, growth factor free medium. Both MSCs and BM-EPCs contributed to these tubes. However, cell proliferation was greatly reduced in co-culture and morphological differences were observed. Gene expression and cluster analysis for wide panel of angiogenesis-related transcripts demonstrated up-regulation of angiogenic markers but down-regulation of many other cytokines. These data suggest that cross-talk occurs in between BM-EPCs and MSCs through paracrine and direct cell contact mechanisms leading to modulation of the angiogenic response.

Ahn, S. Y., et al. (2018). "Vascular endothelial growth factor mediates the therapeutic efficacy of mesenchymal stem cell-derived extracellular vesicles against neonatal hyperoxic lung injury." Exp Mol Med **50**(4): 26.

We previously reported the role of vascular endothelial growth factor (VEGF) secreted by mesenchymal stem cells (MSCs) in protecting against neonatal hyperoxic lung injuries. Recently, the paracrine protective effect of MSCs was reported to be primarily mediated by extracellular vesicle (EV) secretion. However, the therapeutic efficacy of MSC-derived EVs and the role of the VEGF contained within EVs in neonatal hyperoxic lung injury have not been elucidated. The aim of the study was to determine whether MSC-derived EVs attenuate neonatal hyperoxic lung injury and, if so, whether this protection is mediated via the transfer of VEGF. We compared the therapeutic efficacy of MSCs, MSC-derived EVs with or without VEGF knockdown, and fibroblast-derived EVs in vitro with a rat lung epithelial cell line challenged with H2O2 and in vivo with newborn Sprague-Dawley rats exposed to hyperoxia (90%) for 14 days. MSCs (1 x 10(5) cells) or EVs (20 microg) were administered intratracheally on postnatal day 5. The MSCs and MSC-derived EVs, but not the EVs derived from VEGF-knockdown MSCs or fibroblasts, attenuated the in vitro H2O2-induced L2 cell death and the in vivo hyperoxic lung injuries, such as impaired alveolarization and angiogenesis, increased cell death, and activated macrophages and proinflammatory cytokines. PKH67-stained EVs were internalized into vascular pericytes (22.7%), macrophages (21.3%), type 2 epithelial cells (19.5%), and fibroblasts (4.4%) but not into vascular endothelial cells. MSC-derived EVs are as effective as parental MSCs for attenuating neonatal hyperoxic lung injuries, and this protection was mediated primarily by the transfer of VEGF.

Ai, W. J., et al. (2015). "R-Smad signaling-mediated VEGF expression coordinately regulates endothelial cell differentiation of rat mesenchymal stem cells." Stem Cells Dev **24**(11): 1320-1331.

A low-efficiency yield hinders the use of stem cells as a source of endothelial cells (ECs) for therapeutic vascularization, and the diversity of the transforming growth factor-beta (TGF-beta) superfamily has undermined understanding the effects of its potent vascularization-inducing. Herein, we studied the role of the TGF-beta superfamily in EC differentiation of rat bone marrow mesenchymal stem cells (MSCs) induced by Smad2/3 and Smad1/5/8 signaling. MSCs that had been sorted by flow cytometry as CD31-negative were cultured for 14 days in medium supplemented with vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) as the control. The Smad2/3 pathway was activated by TGF-beta1 and Smad1/5/8 by bone morphogenetic proteins (BMPs). In the early phase in the Smad2/3-activated group, there were 10% CD31-positive cells, which was significantly higher than in the control group. A low Smad1/5/8 phosphorylation level after BMP4 activation doubled the number of CD31-positive cells, while a higher phosphorylation level after BMP9 activation showed no effect. A Smad2/3 inhibitor initially blocked differentiation but later promoted it, while a Smad1/5/8 inhibitor reversed the induction observed with BMPs. Moreover, the positive effects of R-Smad on differentiation were weakened by the VEGF neutralizing antibody, and a Smad3 inhibitor decreased VEGF

expression and blocked differentiation in both the early and late phases. In conclusion, differentiation of ECs from MSCs via Smad2/3 signaling is stage dependent. Activation, particularly by Smad3, significantly promotes differentiation at an early phase but later is suppressive. A low Smad1/5/8 phosphorylation level has a positive effect, and R-Smad effects are partly mediated by VEGF.

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

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

Al-Sowayan, B., et al. (2017). "The effect of endothelial cell activation and hypoxia on placental chorionic mesenchymal stem/stromal cell migration." Placenta **59**: 131-138.

INTRODUCTION: Chorionic mesenchymal stem/stromal cells (CMSC) can be isolated from the placenta in large numbers. Although their functions are yet to be fully elucidated, they have a role in tissue development and repair. To fulfil such a role, CMSC must be able to migrate to the microenvironment of the injury site. This process is not fully understood and the aim of this study therefore, was to examine in vitro CMSC migration in response to tissue inflammation and hypoxic conditioning. METHODS: CMSC were derived from the chorionic villi. A trans-endothelium migration (TEM) assay was used to study CMSC migration through an activated endothelial cell monolayer using the HMEC-1 cell line. A cytokine array was used to identify and compare the cytokine production profile of activated versus non-activated HMEC-1. RESULTS: There were significant changes in cytokine production by HMEC-1 cells following lipopolysaccharide (LPS) treatment and hypoxic conditioning. Despite this, results from the TEM assay showed no significant change in the average number of CMSC that migrated through the LPS activated HMEC-1 layer compared to the untreated control. Furthermore, there was no significant change in the average number of CMSC that migrated through the HMEC-1 monolayer when exposed to hypoxic (1% O2), normoxic (8% O2) or hyperoxic (21% O2) conditions. CONCLUSION: These data suggest that cell functions such as transendothelial migration can vary between MSC derived from different tissues in response to the same biological cues.

Antonyshyn, J. A., et al. (2018). "Limited Endothelial Plasticity of Mesenchymal Stem Cells Revealed by Quantitative Phenotypic Comparisons to Representative Endothelial Cell Controls." Stem Cells Transl Med.

Considerable effort has been directed toward deriving endothelial cells (ECs) from adipose-derived mesenchymal stem cells (ASCs) since 2004, when it was first suggested that ECs and adipocytes share a common progenitor. While the capacity of ASCs to express endothelial markers has been repeatedly demonstrated, none constitute conclusive evidence of an endothelial phenotype as all reported markers have been detected in other, non-endothelial cell types. In this study, quantitative phenotypic comparisons to representative EC controls were used to determine the extent of endothelial differentiation being achieved with ASCs. ASCs were harvested from human subcutaneous abdominal white adipose tissue, and their endothelial differentiation was induced using well-established biochemical stimuli. Reverse transcription quantitative real-time polymerase chain reaction and parallel reaction monitoring mass spectrometry were used to quantify their expression of endothelial genes and corresponding proteins, respectively. Flow cytometry was used to quantitatively assess their uptake of acetylated low-density lipoprotein (AcLDL). Human umbilical vein, coronary artery, and dermal microvascular ECs were used as positive controls to reflect the phenotypic heterogeneity between ECs derived from different vascular beds. Biochemically conditioned ASCs were found to upregulate their expression of endothelial genes and proteins, as well as AcLDL uptake, but their abundance remained orders of magnitude lower than that observed in the EC controls despite their global proteomic heterogeneity. The findings of this investigation demonstrate the strikingly limited extent of endothelial differentiation being achieved with ASCs using well-established biochemical stimuli, and underscore the importance of quantitative phenotypic comparisons to representative primary cell controls in studies of differentiation. Stem Cells Translational Medicine 2018.

Baber, S. R., et al. (2007). "Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction." Am J Physiol Heart Circ Physiol **292**(2): H1120-1128.

The administration of mesenchymal stem cells (MSCs) has been proposed for the treatment of pulmonary hypertension. However, the effect of intratracheally administered MSCs on the pulmonary vascular bed in monocrotaline-treated rats has not been determined. In the present study, the effect of intratracheal administration of rat MSCs (rMSCs) on monocrotaline-induced pulmonary hypertension and impaired endothelium-dependent responses were investigated in the rat. Intravenous injection of monocrotaline increased pulmonary arterial pressure and vascular resistance and decreased pulmonary vascular responses to acetylcholine without altering responses to sodium nitroprusside and without altering systemic responses to the vasodilator agents when responses were evaluated at 5 wk. The intratracheal injection of 3 x 10(6) rMSCs 2 wk after administration of monocrotaline attenuated the rise in pulmonary arterial pressure and pulmonary vascular resistance and restored pulmonary responses to acetylcholine toward values measured in control rats. Treatment with rMSCs decreased the right ventricular hypertrophy induced by monocrotaline. Immunohistochemical studies showed widespread distribution of lacZ-labeled rMSCs in lung parenchyma surrounding airways in monocrotaline-treated rats. Immunofluorescence studies revealed that transplanted rMSCs retained expression of von Willebrand factor and smooth muscle actin markers specific for endothelial and smooth muscle phenotypes. However, immunolabeled cells were not detected in the wall of pulmonary vessels. These data suggest that the decrease in pulmonary vascular resistance and improvement in response to acetylcholine an endothelium-dependent vasodilator in monocrotaline-treated rats may result from a paracrine effect of the transplanted rMSCs in lung parenchyma, which improves vascular endothelial function in the monocrotaline-injured lung.

Bourget, J. M., et al. (2016). "Patterning of Endothelial Cells and Mesenchymal Stem Cells by Laser-Assisted Bioprinting to Study Cell Migration." Biomed Res Int **2016**: 3569843.

Tissue engineering of large organs is currently limited by the lack of potent vascularization in

vitro. Tissue-engineered bone grafts can be prevascularized in vitro using endothelial cells (ECs). The microvascular network architecture could be controlled by printing ECs following a specific pattern. Using laser-assisted bioprinting, we investigated the effect of distance between printed cell islets and the influence of coprinted mesenchymal cells on migration. When printed alone, ECs spread out evenly on the collagen hydrogel, regardless of the distance between cell islets. However, when printed in coculture with mesenchymal cells by laser-assisted bioprinting, they remained in the printed area. Therefore, the presence of mesenchymal cell is mandatory in order to create a pattern that will be conserved over time. This work describes an interesting approach to study cell migration that could be reproduced to study the effect of trophic factors.

Boyd, N. L., et al. (2013). "Dissecting the role of human embryonic stem cell-derived mesenchymal cells in human umbilical vein endothelial cell network stabilization in three-dimensional environments." Tissue Eng Part A **19**(1-2): 211-223.

The microvasculature is principally composed of two cell types: endothelium and mural support cells. Multiple sources are available for human endothelial cells (ECs) but sources for human microvascular mural cells (MCs) are limited. We derived multipotent mesenchymal progenitor cells from human embryonic stem cells (hES-MC) that can function as an MC and stabilize human EC networks in three-dimensional (3D) collagen-fibronectin culture by paracrine mechanisms. Here, we have investigated the basis for hES-MC-mediated stabilization and identified the pleiotropic growth factor hepatocyte growth factor/scatter factor (HGF/SF) as a putative hES-MC-derived regulator of EC network stabilization in 3D in vitro culture. Pharmacological inhibition of the HGF receptor (Met) (1 mum SU11274) inhibits EC network formation in the presence of hES-MC. hES-MC produce and release HGF while human umbilical vein endothelial cells (HUVEC) do not. When HUVEC are cultured alone the networks collapse, but in the presence of recombinant human HGF or conditioned media from human HGF-transduced cells significantly more networks persist. In addition, HUVEC transduced to constitutively express human HGF also form stable networks by autocrine mechanisms. By enzyme-linked immunosorbent assay, the coculture media were enriched in both angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), but at significantly different levels (Ang1=159+/-15 pg/mL vs. Ang2=30,867+/-2685 pg/mL) contributed by hES-MC and HUVEC, respectively. Although the coculture cells formed stabile network architectures, their morphology suggests the assembly of an immature plexus. When HUVEC and hES-MC were implanted subcutaneously in immune compromised Rag1 mice, hES-MC increased their contact with HUVEC along the axis of the vessel. This data suggests that HUVEC and hES-MC form an immature plexus mediated in part by HGF and angiopoietins that is capable of maturation under the correct environmental conditions (e.g., in vivo). Therefore, hES-MC can function as microvascular MCs and may be a useful cell source for testing EC-MC interactions.

Brunt, K. R., et al. (2007). "Endothelial progenitor cell and mesenchymal stem cell isolation, characterization, viral transduction." Methods Mol Med **139**: 197-210.

Endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) have emerged as potentially useful substrates for neovascularization and tissue repair and bioengineering. EPCs are a heterogeneous group of endothelial cell precursors originating in the hematopoietic compartment of the bone marrow. MSCs are a rare population of fibroblast-like cells derived from the bone marrow stroma, constituting approximately 0.001-0.01% of the nucleated cells in the marrow. Both cells types have been isolated from the bone marrow. In addition, EPC can be isolated from peripheral blood as well as the spleen, and MSC has also been isolated from peripheral adipose tissue. Several approaches have been used for the isolation of EPC and MSC, including density centrifugation and magnetic bead selection. Phenotypic characterization of both cell types is carried out using immunohistochemical detection and fluorescence-activated cell sorting analysis of cell-surface molecule expression. However, the lack of specific markers for each cell type renders their characterization difficult and ambiguous. In this chapter, we describe the methods that we use routinely for isolation, characterization, and genetic modification of EPC and MSC

from human, rabbit, and mouse peripheral blood and bone marrow.

Cipriani, P., et al. (2007). "Impairment of endothelial cell differentiation from bone marrow-derived mesenchymal stem cells: new insight into the pathogenesis of systemic sclerosis." Arthritis Rheum **56**(6): 1994-2004.

OBJECTIVE: Systemic sclerosis (SSc) is a disorder characterized by vascular damage and fibrosis of the skin and internal organs. Despite marked tissue hypoxia, there is no evidence of compensatory angiogenesis. The ability of mesenchymal stem cells (MSCs) to differentiate into endothelial cells was recently demonstrated. The aim of this study was to determine whether impaired differentiation of MSCs into endothelial cells in SSc might contribute to disease pathogenesis by decreasing endothelial repair. METHODS: MSCs obtained from 7 SSc patients and 15 healthy controls were characterized. The number of colony-forming unit-fibroblastoid colonies was determined. After culture in endothelial-specific medium, the endothelial-like MSC (EL-MSC) phenotype was assessed according to the surface expression of vascular endothelial growth factor receptors (VEGFRs). Senescence, chemoinvasion, and capillary morphogenesis studies were also performed. RESULTS: MSCs from SSc patients displayed the same phenotype and clonogenic activity as those from controls. In SSc MSCs, a decreased percentage of VEGFR-2+, CXCR4+, VEGFR-2+/CXCR4+ cells and early senescence was detected. After culturing, SSc EL-MSCs showed increased expression of VEGFR-1, VEGFR-2, and CXCR4, did not express CD31 or annexin V, and showed significantly decreased migration after specific stimuli. Moreover, the addition of VEGF and stromal cell-derived factor 1 to cultured SSc EL-MSCs increased their angiogenic potential less than that in controls. CONCLUSION: Our data strongly suggest that endothelial repair may be affected in SSc. The possibility that endothelial progenitor cells could be used to increase vessel growth in chronic ischemic tissues may open up new avenues in the treatment of vascular damage caused by SSc.

De Luca, A., et al. (2012). "Mesenchymal stem cell-derived interleukin-6 and vascular endothelial growth factor promote breast cancer cell migration." J Cell Biochem **113**(11): 3363-3370.

Several different cytokines and growth factors secreted by mesenchymal stem cells (MSCs) have been hypothesized to play a role in breast cancer progression. By using a small panel of breast cancer cell lines (MCF-7, T47D, and SK-Br-3 cells), we analyzed the role of interleukin-6 (IL-6) and vascular endothelial growth factor A (VEGF) in the cross-talk between MSCs and breast cancer cells. We performed migration assays in which breast cancer cells were allowed to migrate in response to conditioned medium from MSCs (MSCs-CM), in absence or in presence of the anti-VEGF antibody bevacizumab or an anti-IL-6 antibody, alone or in combination. We found that anti-VEGF and anti-IL-6 antibodies inhibited the migration of breast cancer cells and that the combination had an higher inhibitory effect. We next evaluated the effects of recombinant VEGF and IL-6 proteins on breast cancer cell growth and migration. IL-6 and VEGF had not significant effects on the proliferation of breast carcinoma cells. In contrast, both VEGF and IL-6 significantly increased the ability to migrate of MCF-7, T47D and SK-Br-3 cells, with the combination showing a greater effect as compared with treatment with a single protein. The combination of VEGF and IL-6 produced in breast cancer cells a more significant and more persistent activation of MAPK, AKT, and p38MAPK intracellular signaling pathways. These results suggest that MSC-secreted IL-6 and VEGF may act as paracrine factors to sustain breast cancer cell migration.

de Oliveira, L. F., et al. (2015). "Priming Mesenchymal Stem Cells with Endothelial Growth Medium Boosts Stem Cell Therapy for Systemic Arterial Hypertension." Stem Cells Int **2015**: 685383.

Systemic arterial hypertension (SAH), a clinical syndrome characterized by persistent elevation of arterial pressure, is often associated with abnormalities such as microvascular rarefaction, defective angiogenesis, and endothelial dysfunction. Mesenchymal stem cells (MSCs), which normally induce angiogenesis and improve endothelial function, are defective in SAH. The central aim of this study was to evaluate whether priming of MSCs with endothelial growth medium (EGM-2) increases their therapeutic effects in spontaneously

hypertensive rats (SHRs). Adult female SHRs were administered an intraperitoneal injection of vehicle solution (n = 10), MSCs cultured in conventional medium (DMEM plus 10% FBS, n = 11), or MSCs cultured in conventional medium followed by 72 hours in EGM-2 (pMSC, n = 10). Priming of the MSCs reduced the basal cell death rate in vitro. The administration of pMSCs significantly induced a prolonged reduction (10 days) in arterial pressure, a decrease in cardiac hypertrophy, an improvement in endothelium-dependent vasodilation response to acetylcholine, and an increase in skeletal muscle microvascular density compared to the vehicle and MSC groups. The transplanted cells were rarely found in the hearts and kidneys. Taken together, our findings indicate that priming of MSCs boosts stem cell therapy for the treatment of SAH.

Deezagi, A. and S. Shomali (2018). "Prostaglandin F-2alpha Stimulates The Secretion of Vascular Endothelial Growth Factor and Induces Cell Proliferation and Migration of Adipose Tissue Derived Mesenchymal Stem Cells." Cell J **20**(2): 259-266.

OBJECTIVES: Tissue engineering today uses factors that can induce differentiation of mesenchymal stem cells (MSCs) into other cell types. However, the problem of angiogenesis in this differentiated tissue remains an unresolved area of research interest. The aim of this study was to investigate the effects of prostaglandin F-2alpha (PGF-2alpha) on the expression of vascular endothelial growth factor (VEGF) in human adipose tissue derived MSCs. MATERIALS AND METHODS: In this experimental research, human adipose tissue was digested using collagenase. The isolated MSCs cells were treated with PGF-2alpha (up to 5 mug/ml) and incubated for 96 hours. Cell proliferation, secretion of VEGF and cell migration were spontaneously assayed by MTT, BrdU, ELISA, RT-PCR and scratching methods. RESULTS: Cell growth at 1.0, 2.5, 5 mug/ml of PGF-2alpha was not significantly reduced compared to control cells, suggesting that these concentrations of PGF-2alpha are not toxic to cell growth. The results of the BrdU incorporation assay indicated that, in comparison to untreated cells, BrdU incorporation was respectively 1.08, 1.96, 2.0 and 1.8 fold among cells treated with 0.1, 1.0, 2.5 and 5.0 mug/ml of PGF-2alpha. The scratching test also demonstrated a positive influence on cell proliferation and migration. Cells treated with 1.0 mug/ml of PGF-2alpha for 12 hours showed the highest relative migration and coverage in comparison to untreated cells. Quantitative VEGF ELISA and RTPCR results indicated an increase in VEGF expression and secretion in the presence of PGF-2alpha. The amount of VEGF produced in response to 0.1, 1.0, 2.5 and 5.0 mug/ml of PGF-2alpha was 62.4 +/- 3.2 , 66.3 +/- 3.7, 53.1 +/- 2.6 and 49.0 +/- 2.3 pg/ml, respectively, compared to the 35.2 +/- 2.1 pg/ml produced by untreated cells. CONCLUSIONS: Stimulation of VEGF secretion by PGF-2alpha treated MSCs could be useful for the induction of angiogenesis in tissue engineering in vitro.

Deuse, T., et al. (2009). "Hepatocyte growth factor or vascular endothelial growth factor gene transfer maximizes mesenchymal stem cell-based myocardial salvage after acute myocardial infarction." Circulation **120**(11 Suppl): S247-254.

BACKGROUND: Mesenchymal stem cell (MSC)-based regenerative strategies were investigated to treat acute myocardial infarction and improve left ventricular function. METHODS AND RESULTS: Murine AMI was induced by coronary ligation with subsequent injection of MSCs, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), or MSCs +HGF/VEGF into the border zone. Left ventricular ejection fraction was calculated using micro-computed tomography imaging after 6 months. HGF and VEGF protein injection (with or without concomitant MSC injection) significantly and similarly improved the left ventricular ejection fraction and reduced scar size compared with the MSC group, suggesting that myocardial recovery was due to the cytokines rather than myocardial regeneration. To provide sustained paracrine effects, HGF or VEGF overexpressing MSCs were generated (MSC-HGF, MSC-VEGF). MSC-HGF and MSC-VEGF showed significantly increased in vitro proliferation and increased in vivo proliferation within the border zone. Cytokine production correlated with MSC survival. MSC-HGF- and MSC-VEGF-treated animals showed smaller scar sizes, increased peri-infarct vessel densities, and better preserved left ventricular function when compared with MSCs transfected with empty vector. Murine cardiomyocytes were exposed to hypoxic in

vitro conditions. The LDH release was reduced, fewer cardiomyocytes were apoptotic, and Akt activity was increased if cardiomyocytes were maintained in conditioned medium obtained from MSC-HGF or MSC-VEGF cultures. CONCLUSIONS: This study showed that (1) elevating the tissue levels of HGF and VEGF after acute myocardial infarction seems to be a promising reparative therapeutic approach, (2) HGF and VEGF are cardioprotective by increasing the tolerance of cardiomyocytes to ischemia, reducing cardiomyocyte apoptosis and increasing prosurvival Akt activation, and (3) MSC-HGF and MSC-VEGF are a valuable source for increased cytokine production and maximize the beneficial effect of MSC-based repair strategies.

Du, P., et al. (2016). "Elasticity Modulation of Fibroblast-Derived Matrix for Endothelial Cell Vascular Morphogenesis and Mesenchymal Stem Cell Differentiation." Tissue Eng Part A **22**(5-6): 415-426.

Biophysical properties of the microenvironment, including matrix elasticity and topography, are known to affect various cell behaviors; however, the specific role of each factor is unclear. In this study, fibroblast-derived matrix (FDM) was used as cell culture substrate and physically modified to investigate the influence of its biophysical property changes on human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (hMSCs) behavior in vitro. These FDMs were physically modified by simply storing them at different temperatures: the one stored at 4 degrees C, maintained its original properties, was considered natural FDM, whereas the ones stored at -20 degrees C or -80 degrees C, exhibited a distinct surface morphology, were considered physically modified FDM. Physical modification induced matrix fiber rearrangement in FDM, forming different microstructures on the surface as characterized by focused ion beam (FIB)-cryoSEM. A significant increase of matrix elasticity was found with physically modified FDMs as determined by atomic force microscopy. HUVEC and hMSC behaviors on these natural and physically modified FDMs were observed and compared with each other and with gelatin-coated coverslips. HUVECs showed a similar adhesion level on these substrates at 3 h, but exhibited different proliferation rates and morphologies at 24 h; HUVECs on natural FDM proliferated relatively slower and assembled to capillary-like structures (CLSs). It is observed that HUVECs assembled to CLSs on natural FDMs are independent on the exogenous growth factors and yet dependent on nonmuscle myosin II activity. This result indicates the important role of matrix mechanical properties in regulating HUVECs vascular morphogenesis. As for hMSCs multilineage differentiation, adipogenesis is improved on natural FDM that with lower matrix elasticity, while osteogenesis is accelerated on physically modified FDMs that with higher matrix elasticity, these results further confirm the crucial role of matrix elasticity on cell fate determination.

Duttenhoefer, F., et al. (2015). "Endothelial Progenitor Cell Fraction Contained in Bone Marrow-Derived Mesenchymal Stem Cell Populations Impairs Osteogenic Differentiation." Biomed Res Int **2015**: 659542.

In bone tissue engineering (TE) endothelial cell-osteoblast cocultures are known to induce synergies of cell differentiation and activity. Bone marrow mononucleated cells (BMCs) are a rich source of mesenchymal stem cells (MSCs) able to develop an osteogenic phenotype. Endothelial progenitor cells (EPCs) are also present within BMC. In this study we investigate the effect of EPCs present in the BMC population on MSCs osteogenic differentiation. Human BMCs were isolated and separated into two populations. The MSC population was selected through plastic adhesion capacity. EPCs (CD34(+) and CD133(+)) were removed from the BMC population and the resulting population was named depleted MSCs. Both populations were cultured over 28 days in osteogenic medium (Dex(+)) or medium containing platelet lysate (PL). MSC population grew faster than depleted MSCs in both media, and PL containing medium accelerated the proliferation for both populations. Cell differentiation was much higher in Dex(+) medium in both cases. Real-time RT-PCR revealed upregulation of osteogenic marker genes in depleted MSCs. Higher values of ALP activity and matrix mineralization analyses confirmed these results. Our study advocates that absence of EPCs in the MSC population enables higher osteogenic gene expression and matrix mineralization and

therefore may lead to advanced bone neoformation necessary for TE constructs.

Eren, F., et al. (2016). "Targeted mesenchymal stem cell and vascular endothelial growth factor strategies for repair of nerve defects with nerve tissue implanted autogenous vein graft conduits." Microsurgery **36**(7): 578-585.

Peripheral nerve gaps exceeding 1 cm require a bridging repair strategy. Clinical feasibility of autogenous nerve grafting is limited by donor site comorbidity. In this study we investigated neuroregenerative efficacy of autogenous vein grafts implanted with tissue fragments from distal nerve in combination with vascular endothelial growth factor (VEGF) or mesenchymal stem cells (MSCs) in repair of rat peripheral nerve defects. Six-groups of Sprague-Dawley rats (n = 8 each) were evaluated in the autogenous setting using a 1.6 cm long peroneal nerve defect: Empty vein graft (group 1), Nerve graft (group 2), Vein graft and nerve fragments (group 3), Vein graft and nerve fragments and blank microspheres (group 4), Vein graft and nerve fragments and VEGF microspheres (group 5), Vein graft and nerve fragments and MSCs (group 6). Nerve fragments were derived from distal segment. Walking track analysis, electrophysiology and nerve histomorphometry were performed for assessment. Peroneal function indices (PFI), electrophysiology (amplitude) and axon count results for group 2 were -9.12 +/- 3.07, 12.81 +/- 2.46 mV, and 1697.88 +/- 166.18, whereas the results for group 5 were -9.35 +/- 2.55, 12.68 +/- 1.78, and 1566 +/- 131.44, respectively. The assessment results did not reveal statistical difference between groups 2 and 5 (P > 0.05). The best outcomes were seen in group 2 and 5 followed by group 6. Compared to other groups, poorest outcomes were seen in group 1 (P </= 0.05). PFI, electrophysiology (amplitude) and axon count results for group 1 were -208.82 +/- 110.69, 0.86 +/- 0.52, and 444.50 +/- 274.03, respectively. Vein conduits implanted with distal nerve-derived nerve fragments improved axonal regeneration. VEGF was superior to MSCs in facilitating nerve regeneration. (c) 2015 Wiley Periodicals, Inc. Microsurgery 36:578-585, 2016.

Fang, L. J., et al. (2010). "[Expression of MMP-2 during differentiation of vascular endothelial cell from bone marrow mesenchymal stem cell in porcine]." Shanghai Kou Qiang Yi Xue **19**(3): 270-274.

PURPOSE: To investigate the expression of MMP-2 in porcine bone marrow mesenchymal stem cells (MSCs) in the process of differentiation into vascular endothelial cells in vitro. METHODS: Porcine MSCs were isolated from bone marrow and grown in vitro. At 3-, 7-day after VEGF induction, immunohistochemical and flow cytometrical examinations were carried out to detect the staining of FVIII. The expression of MMP-2 was analyzed by Western blot. RESULTS: At 3-, 7-day after VEGF induction, the morphology of the cells gradually changed. The cells gradually showed a ball-like appearance at 3-day. They connected with adjacent cells and formed tube-like structure after 7 days. The factor VIII-positive cells could be observed at 3-day after VEGF induction. The positive cells increased and joined to neighboring FVIII-positive cells, formed tube-like structures at 7-day after VEGF treatment. MSCs maintained in control medium stained negative. FACS analysis showed that untreated MSCs were negative for FVIII. The number of FVIII positive cells was not significantly increased at 3-day after induction (3%). But at 7-day after induction, FVIII-positive cells were significantly increased (13%). The expression of MMP-2 was increased as early as 1 hour post VEGF treatment. CONCLUSION: Porcine MSCs have the potential to differentiate into vascular endothelial cells in vitro and this process involves MMP-2.

Feng, Y., et al. (2014). "The involvement of CXCL11 in bone marrow-derived mesenchymal stem cell migration through human brain microvascular endothelial cells." Neurochem Res **39**(4): 700-706.

Bone marrow-derived mesenchymal stem cells (MSCs) transplant into the brain, where they play a potential therapeutic role in neurological diseases. However, the blood-brain barrier (BBB) is a native obstacle for MSCs entry into the brain. Little is known about the mechanism behind MSCs migration across the BBB. In the present study, we modeled the interactions between human MSCs (hMSCs) and human brain microvascular endothelial cells (HBMECs) to mimic the BBB microenvironment. Real-time PCR analysis indicated that the chemokine CXCL11 is produced by hMSCs and the chemokine receptor CXCR3 is expressed on

HBMECs. Further results indicate that CXCL11 secreted by hMSCs may interact with CXCR3 on HBMECs to induce the disassembly of tight junctions through the activation of ERK1/2 signaling in the endothelium, which promotes MSCs transendothelial migration. These findings are relevant for understanding the biological responses of MSCs in BBB environments and helpful for the application of MSCs in neurological diseases.

Gao, C. Q., et al. (2007). "[The experimental studies on cell transplantation into chronic ischemic myocardium using mesenchymal stem cells modified by recombinant adenovirus carrying vascular endothelial growth factors 165 gene]." Zhonghua Wai Ke Za Zhi **45**(14): 990-993.

OBJECTIVE: To observe the therapeutic effect of vascular endothelial growth factors 165 (VEGF165) gene expressing mesenchymal stem cells (MSCs) in chronic myocardial infarction model by providing enhanced cardioprotection, followed by angiogenic effects in infarcted myocardium. METHODS: Recombinant adenovirus vector carrying VEGF165 gene (rAd-VFGF165) was constructed. MSCs were harvested through gradient centrifugation, then were cultivated, multiplied and expanded. Recombinant adenoviruses mediated VEGF165 gene were transfected into MSCs, and the MSCs were labelled by DAPI. The left anterior descending branch of rabbits were ligated to establish a myocardial infarction model; and the animals survived for 6 weeks were randomly divided into three groups: VEGF165-expressing MSCs transplanted (Group I), MSCs transplanted (Group II) and dulbecco modified eagles medium injected (Group III). At 4 weeks after cell transplantation, the MSCs were detected by DAPI staining in infarcted region. The cardiac functions were estimated by UCG. The microvascular density in infarcted area were estimated through CD34 immunohistochemical analysis. RESULTS: Four weeks after cell transplantation, ejection fraction, E wave/A wave ratio and capillary density of the infarcted region were most improved in Group I compared with Group II and control group ($P < 0.05$). DAPI positive cells were most increased in Group I. CONCLUSIONS: The transplantation of VEGF165-expressing MSCs had a better therapeutic effect than the transplantation of simplex MSCs. This combined strategy of MSCs transplantation with vgene therapy could be a useful therapy for the treatment of myocardial infarction.

Gao, F., et al. (2007). "A promising strategy for the treatment of ischemic heart disease: Mesenchymal stem cell-mediated vascular endothelial growth factor gene transfer in rats." Can J Cardiol **23**(11): 891-898.

BACKGROUND: Treatment of ischemic heart disease (IHD) remains a worldwide problem. Gene therapy, and recently, cell transplantation, have made desirable progress. A combination of appropriate stem cells and angiogenic genes appears promising in treating IHD. OBJECTIVE: To study the results of angiogenesis and myogenesis induced by transplantation of the adenovirus carrying human vascular endothelial growth factor 165 (Ad-hVEGF(165))-transfected mesenchymal stem cells (MSCs) in IHD compared with direct MSC transplantation or Ad-hVEGF165 delivery. METHODS: Cultured MSCs were transfected by Ad-hVEGF(165), and secreted VEGF was measured by ELISA in vitro. Ad-hVEGF(165)-transfected MSCs (MSC/VEGF group), MSCs (MSC group), Ad-hVEGF(165) (VEGF group) or a serum-free medium (control group) was injected into syngeneic Wistar rats immediately after left coronary artery occlusion. All cells were marked with CM-DiI (Molecular Probes, USA) before transplantation. One week after treatment, messenger RNA expression of hVEGF(165) in the MSC/VEGF group was found to be significantly higher than in other groups by reverse transcriptase-polymerase chain reaction analysis. One month after cell transplantation, left ventricular (LV) ejection fraction, capillary density of the infarcted region, infarct size and hemodynamic parameters (including LV end-diastolic pressure, LV+dP/dt and LV-dP/dt) were measured and immunohistochemical analysis was performed. RESULTS: A high level of VEGF was expressed by Ad-hVEGF(165)-transfected MSCs. LV ejection fraction, mean capillary density of the infarcted region and hemodynamic parameters were significantly improved in the MSC/VEGF group compared with the MSC group, the VEGF group and the control group ($P<0.001$ for all). Partly transplanted MSCs showed the cardiomyocyte phenotype, expressed desmin and cardiac troponin T, and resulted in angiogenesis in the

ischemic myocardium. However, a few transplanted MSCs incorporated into the vascular structure and most of the new vascular components were host-derived. CONCLUSIONS: The combined strategy of MSC transplantation and VEGF gene therapy can produce effective myogenesis and host-derived angiogenesis, resulting in the prevention of progressive heart dysfunction after myocardial infarction.

Gao, Z., et al. (2012). "Vascular endothelial growth factor participates in modulating the C6 glioma-induced migration of rat bone marrow-derived mesenchymal stem cells and upregulates their vascular cell adhesion molecule-1 expression." Exp Ther Med **4**(6): 993-998.

Bone marrow-derived mesenchymal stem cells (BMSCs) have been shown to be able to migrate towards glioma, but the molecular mechanisms responsible for this migratory behavior still require further elucidation. This study aimed to test the role of vascular endothelial growth factor (VEGF) in the C6 glioma-induced migration of BMSCs, evaluate the effect of VEGF on the migratory capacity and vascular cell adhesion molecule-1 (VCAM-1) expression of BMSCs and explore the role of VCAM-1 in the VEGF-induced migration of BMSCs. The results showed that C6 glioma cells significantly increased the migration of BMSCs in vitro, which was partially blocked by a VEGF neutralizing antibody, and 20 ng/ml recombinant rat VEGF(164) incubation enhanced the migration of BMSCs. Moreover, 12 h of 20 ng/ml VEGF(164) incubation upregulated the VCAM-1 expression of BMSCs and the blocking of VCAM-1 reduced the VEGF(164)-induced migration of BMSCs. The data also revealed that LY294002, an inhibitor of phosphoinositide-3-kinase (PI3K), decreased the VEGF-induced migration and VCAM-1 expression of BMSCs. These findings indicate that VEGF participates in mediating the C6 glioma-induced migration of BMSCs by upregulating their VCAM-1 expression, and that PI3K is involved in the signal transduction of VEGF(164)-induced migration and VCAM-1 expression of BMSCs.

Ghem, C., et al. (2017). "Combined Analysis of Endothelial, Hematopoietic, and Mesenchymal Stem Cell Compartments Shows Simultaneous but Independent Effects of Age and Heart Disease." Stem Cells Int **2017**: 5237634.

Clinical trials using stem cell therapy for heart diseases have not reproduced the initial positive results obtained with animal models. This might be explained by a decreased regenerative capacity of stem cells collected from the patients. This work aimed at the simultaneous investigation of endothelial stem/progenitor cells (EPCs), mesenchymal stem/progenitor cells (MSCs), and hematopoietic stem/progenitor cells (HSCs) in sternal bone marrow samples of patients with ischemic or valvular heart disease, using flow cytometry and colony assays. The study included 36 patients referred for coronary artery bypass grafting or valve replacement surgery. A decreased frequency of stem cells was observed in both groups of patients. Left ventricular dysfunction, diabetes, and intermediate risk in EuroSCORE and SYNTAX score were associated with lower EPCs frequency, and the use of aspirin and beta-blockers correlated with a higher frequency of HSCs and EPCs, respectively. Most importantly, the distribution of frequencies in the three stem cell compartments showed independent patterns. The combined investigation of the three stem cell compartments in patients with cardiovascular diseases showed that they are independently affected by the disease, suggesting the investigation of prognostic factors that may be used to determine when autologous stem cells may be used in cell therapy.

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

Several studies have reported the benefits of mesenchymal stem cells (MSCs) for bone tissue engineering. However, vascularization remains one of the main obstacles that must be overcome to reconstruct large bone defects. In vitro prevascularization of the three-dimensional (3-D) constructs using co-cultures of human progenitor-derived endothelial cells (PDECs) with human bone marrow mesenchymal stem cells (HBMSCs) appeared as a potential strategy. However, the crosstalk between the two lineages has been studied in two-dimensional (2-D), but remains unknown in 3-D. The aim of this study is to investigate the cell interactions between PDECs and HBMSCs in a porous matrix

composed of polysaccharides. This biodegradable scaffold promotes cell interactions by inducing multicellular aggregates composed of HBMSCs surrounded by PDECs. Cell aggregation contributes to the formation of junctional proteins composed of Connexin43 (Cx43) and VE-cadherin, and an activation of osteoblastic differentiation of HBMSCs stimulated by the presence of PDECs. Inhibition of Cx43 by mimetic peptide 43GAP27 induced a decrease in mRNA levels of Cx43 and all the bone-specific markers. Finally, subcutaneous implantations for 3 and 8 weeks in NOG mice revealed an increase in osteoid formation with the tissue-engineered constructs seeded with HBMSCs/PDECs compared with those loaded with HBMSCs alone. Taking together, these results demonstrate that this 3-D microenvironment favored cell communication, osteogenesis and bone formation.

He, J., et al. (2012). "Bioceramic-mediated trophic factor secretion by mesenchymal stem cells enhances in vitro endothelial cell persistence and in vivo angiogenesis." Tissue Eng Part A **18**(13-14): 1520-1528.

Mesenchymal stem cells (MSCs) seeded in composite implants formed of hydroxyapatite (HA) and poly (lactide-co-glycolide) (PLG) exhibit increased osteogenesis and enhanced angiogenic potential. Endothelial colony-forming cells (ECFCs) can participate in de novo vessel formation when implanted in vivo. The aim of this study was to determine the capacity of HA-PLG composites to cotransplant MSCs and ECFCs, with the goal of accelerating vascularization and resultant bone formation. The incorporation of HA into PLG scaffolds improved the efficiency of cell seeding and ECFC survival in vitro. We observed increases in mRNA expression and secretion of potent angiogenic factors by MSCs when cultured on HA-PLG scaffolds compared to PLG controls. Upon implantation into an orthotopic calvarial defect, ECFC survival on composite scaffolds was not increased in the presence of MSCs, nor did the addition of ECFCs enhance vascularization beyond increases observed with MSCs alone. Microcomputed tomography (micro-CT) performed on explanted calvarial tissues after 12 weeks revealed no significant differences between treatment groups for bone volume fraction (BVF) or bone mineral density (BMD). Taken together, these results provide evidence that HA-containing composite scaffolds seeded with MSCs can enhance neovascularization, yet MSC-secreted trophic factors do not consistently increase the persistence of co-transplanted ECFCs.

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

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

Herrmann, J. L., et al. (2011). "IL-6 and TGF-alpha costimulate mesenchymal stem cell vascular endothelial growth factor production by ERK-, JNK-, and PI3K-mediated mechanisms." Shock **35**(5): 512-516.

Mesenchymal stem cells (MSCs) protect ischemic tissues in part through paracrine growth factor production. IL-6, which is

upregulated in the heart during ischemia, has been shown to enhance stem cell proliferation and migration. The effect of IL-6 on MSC paracrine function, however, remains unknown. In addition, TGF-alpha increases MSC vascular endothelial growth factor (VEGF) production and may share downstream signaling pathways with IL-6 involving ERK, JNK, and PI3K. We hypothesize that cotreatment with IL-6 and TGF-alpha will result in greater MSC VEGF production than by either treatment alone via these signaling pathways. Murine MSCs were treated with IL-6 (0.05 ng/mL) with or without TGF-alpha (250 ng/mL) and in combination with inhibitors of ERKI/II, JNK, and PI3K for 24 h. Vascular endothelial growth factor concentrations in the supernatants were measured using enzyme-linked immunosorbent assay. Phosphorylation of ERK, JNK, and PI3K was measured using Western blot analysis. IL-6 increased MSC VEGF production at a dose of 0.05 ng/mL, and the combination of IL-6 and TGF-alpha (250 ng/mL) increased VEGF production to a greater extent than IL-6 or TGF-alpha alone. IL-6 induced phosphorylation of ERK, JNK, and PI3K, and inhibition of each suppressed IL-6-induced VEGF production. TGF-alpha cotreatment overcame VEGF suppression after ERK2 inhibition but not ERK1, JNK, or PI3K. These data suggest that IL-6 stimulates MSC VEGF production alone and additively with TGF-alpha via ERK-, JNK-, and PI3K-mediated mechanisms. IL-6 and TGF-alpha cotreatment may be a useful strategy for enhancing MSC VEGF production and cardioprotection during myocardial ischemia.

Hou, J., et al. (2017). "Mesenchymal stem cells promote endothelial progenitor cell proliferation by secreting insulinlike growth factor1." Mol Med Rep **16**(2): 1502-1508.

Bone marrow mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) interact with each other. EPCs are able to promote the selfrenewal of MSCs as niche cells in murine bone marrow, and MSCs are able to promote EPC proliferation in vitro in a coculture system. It has previously been reported that MSCs can secrete insulinlike growth factor1 (IGF1), which serves critical functions in EPC proliferation. However, the mechanism underlying the IGF1mediated proliferation of EPCs remains unclear. The aim of the present study was to reveal the molecular mechanisms regulating this process. The effects of IGF1, which is secreted by MSCs, on EPC proliferation via the PI3K/Akt signaling pathway were examined by MTT assay, reverse transcriptionquantitative polymerase chain reaction and western blot analysis. The present study treated EPCs with various concentrations of IGF1. The results demonstrated that IGF1 significantly induced the proliferation of cultured EPCs. However, this effect was offset by treatment with the phosphatidylinositol 3kinase (PI3K) inhibitor LY294002. These results indicated that the proproliferative effects of IGF1 are mediated in response to the PI3K/protein kinase B signaling pathway.

Hsu, S. H., et al. (2014). "Substrate-dependent modulation of 3D spheroid morphology self-assembled in mesenchymal stem cell-endothelial progenitor cell coculture." Biomaterials **35**(26): 7295-7307.

The structural evolution of three-dimensional spheroids self-assembled from two different types of cells on selective biomaterials is demonstrated in this study. The two types of cells involved in the self-assembly are human mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs). When seeded in different population ratios, they can create a variety of cellular patterns on different biomaterial substrates. When the two populations are matched in initial numbers, they are self-assembled in co-spheroids with different morphologies (i.e. randomly mixed, bumped, or concentric spheroids). The morphologies are influenced by the specific cell-substrate interaction possibly through integrin signaling, as well as a substrate-dependent regulation of heterophilic cell-cell interaction possibly through Notch signaling. In particular, the self-assembled core-shell concentric spheroids from adipose-derived MSCs and EPCs show a greater angiogenic effect in vitro. This study reveals the possibility to modulate the self-assembled morphology as well as the effect of cocultured cells by changing the cell culture substratum.

Hu, S., et al. (2018). "Mesenchymal Stem Cell Microvesicles Restore Protein Permeability Across Primary Cultures of Injured Human Lung Microvascular Endothelial Cells." Stem Cells Transl Med **7**(8): 615-624.

Our previous study demonstrated that mesenchymal stem cell (MSC) microvesicles (MV) reduced lung inflammation, protein

permeability, and pulmonary edema in endotoxin-induced acute lung injury in mice. However, the underlying mechanisms for restoring lung protein permeability were not fully understood. In this current study, we hypothesized that MSC MV would restore protein permeability across injured human lung microvascular endothelial cells (HLMVEC) in part through the transfer of angiopoietin-1 (Ang1) mRNA to the injured endothelium. A transwell coculture system was used to study the effect of MSC MV on protein permeability across HLMVECs injured by cytomix, a mixture of IL-1beta, TNF-alpha, and IFN-gamma (50 ng/ml). Our result showed that cytomix significantly increased permeability to FITC-dextran (70 kDa) across HLMVECs over 24 hours. Administration of MSC MVs restored this permeability in a dose dependent manner, which was associated with an increase in Ang1 mRNA and protein secretion in the injured endothelium. This beneficial effect was diminished when MSC MV was pretreated with an anti-CD44 antibody, suggesting that internalization of MV into the HLMVEC was required for the therapeutic effect. Fluorescent microscopy showed that MSC MV largely prevented the reorganization of cytoskeleton protein F-actin into "actin stress fiber" and restored the location of the tight junction protein ZO-1 and adherens junction protein VE-cadherin in injured HLMVECs. Ang1 siRNA pretreatment of MSC MV prior to administration to injured HLMVECs eliminated the therapeutic effect of MV. In summary, MSC MVs restored protein permeability across HLMVEC in part by increasing Ang1 secretion by injured HLMVEC. Stem Cells Translational Medicine 2018;7:615-624.

Hu, X., et al. (2015). "Heparanase released from mesenchymal stem cells activates integrin beta1/HIF-2alpha/Flk-1 signaling and promotes endothelial cell migration and angiogenesis." Stem Cells **33**(6): 1850-1862.

Heparanase plays important roles in tumor angiogenesis. Our previous study demonstrated that hypoxic preconditioning (HPC) enhanced the angiogenic and therapeutic effects of mesenchymal stem cells (MSCs), effects that were paralleled by enhanced heparanase expression. This study was designed to elucidate the role of heparanase in the improved therapeutic properties of HPC-MSCs and to explore underlying mechanisms using an ischemic rat hind limb model. MSCs transfected with heparanase (MSC(hpa) ) or empty vector (MSC(null) ) were delivered by intramuscular injections to ischemic hind limbs. Hind limbs that received MSC(hpa) recovered blood flow more rapidly at 7 days and acquired higher capillary density at 14 days compared with MSC(null) . Conditioned medium from MSC(hpa) increased endothelial cell migration and promoted greater tube formation relative to that from the MSC(null) groups. Vascular endothelial growth factor receptor 2 (VEGFR2, Flk-1) and its downstream signaling pathway (p38MAPK/HSP27) were significantly increased in human umbilical vein endothelial cells (HUVECs) after treatment with MSC(hpa) conditioned medium. Each of these responses was decreased by cocultured with MSC(hpa-KD) conditioned medium. MSC(hpa) conditioned medium activated hypoxia-inducible factor-2alpha (HIF-2alpha) and increased in parallel the transcript level of Flk-1 as determined by chromatin immunoprecipitation-PCR and luciferase assays. Analyses of integrin expression revealed an important role for integrin beta1 in the regulation of HIF-2alpha. All angiogenic effects of MSC(hpa) conditioned medium were abolished by knockdown of integrin beta1, HIF-2alpha, and Flk-1 in HUVECs with selective shRNAs. These findings identify heparanse as a key regulator of angiogenesis by MSCs. We propose a novel pathway wherein heparanse sequentially activates integrin beta1, HIF-2alpha, Flk-1, and p38MAPK/HSP27 with corresponding enhancement of angiogenesis.

Hua, P., et al. (2014). "Cell transplantation into ischemic myocardium using mesenchymal stem cells transfected by vascular endothelial growth factor." Int J Clin Exp Pathol **7**(11): 7782-7788.

AIMS: To investigate the effects of mesenchymal stem cells (MSCs) transplantation combining with vascular endothelial growth factor (VEGF) gene therapy on myocardium rebuilding, angiogenesis, and heart function improvement in rats with myocardial infarction. METHODS: SD rat MSCs were isolated, cultured in vitro, labeled with BrdU and transfected by Ad.VEGF gene. Four weeks after left anterior descending artery was ligated to create rat

myocardial infarction, cardiac function was examined with echocardiography. Rats were randomly divided into four groups (n = 10 in each group): Group I: MSCs/Ad.VEGF implantation; Group II: MSCs implantation; Group III: Ad.VEGF injection; Group IV: Control. MSCs differentiation was observed 4 weeks after transplantation. Immunohistochemistry and angiogenesis were observed. Echocardiography was performed to detect the effects on heart function. RESULTS: MSCs labeled with BrdU could be identified in host hearts in group I and II, most of them positively stained with cTnT antibody. Echocardiography indicated that the improvement of the LVEF value in group I was more significant than that in the other three groups ($P < 0.01$, respectively). Some cells were incorporated into the coronary capillaries in the infarcted region. The capillary density in group I was higher than that in the other three groups ($P < 0.01$, respectively). CONCLUSION: MSCs implantation combining with VEGF gene therapy can obviously repair damaged myocardium and enhance the angiogenesis in ischemic heart tissue.

Igarashi, Y., et al. (2016). "VEGF-C and TGF-beta reciprocally regulate mesenchymal stem cell commitment to differentiation into lymphatic endothelial or osteoblastic phenotypes." Int J Mol Med **37**(4): 1005-1013.

The direction of mesenchymal stem cell (MSC) differentiation is regulated by stimulation with various growth factors and cytokines. We recently established MSC lines, [transforming growth factor-beta (TGF-beta)-responsive SG2 cells, bone morphogenetic protein (BMP)-responsive SG3 cells, and TGF-beta/BMP-non-responsive SG5 cells], derived from the bone marrow of green fluorescent protein-transgenic mice. In this study, to compare gene expression profiles in these MSC lines, we used DNA microarray analysis to characterize the specific gene expression profiles observed in the TGF-beta-responsive SG2 cells. Among the genes that were highly expressed in the SG2 cells, we focused on vascular endothelial growth factor (VEGF) receptor 3 (VEGFR3), the gene product of FMS-like tyrosine kinase 4 (Flt4). We found that VEGF-C, a specific ligand of VEGFR3, significantly induced the cell proliferative activity, migratory ability (as shown by Transwell migration assay), as well as the phosphorylation of extracellular signal-regulated kinase (ERK)1/2 in the SG2 cells. Additionally, VEGF-C significantly increased the expression of prospero homeobox 1 (Prox1) and lymphatic vessel endothelial hyaluronan receptor 1 (Lyve1), which are lymphatic endothelial cell markers, and decreased the expression of osteogenic differentiation marker genes in these cells. By contrast, TGF-beta significantly increased the expression of early-phase osteogenic differentiation marker genes in the SG2 cells and markedly decreased the expression of lymphatic endothelial cell markers. The findings of our study strongly suggest the following: i) that VEGF-C promotes the proliferative activity and migratory ability of MSCs; and ii) VEGF-C and TGF-beta reciprocally regulate MSC commitment to differentiation into lymphatic endothelial or osteoblastic phenotypes, respectively. Our findings provide new insight into the molecular mechanisms underlying the regenerative ability of MSCs.

Jiang, Y. C., et al. (2018). "Polycaprolactone Nanofibers Containing Vascular Endothelial Growth Factor-Encapsulated Gelatin Particles Enhance Mesenchymal Stem Cell Differentiation and Angiogenesis of Endothelial Cells." Biomacromolecules **19**(9): 3747-3753.

During the regeneration of tissues and organs, growth factors (GFs) play a vital role by affecting cell behavior. However, because of the low half-life time and quick degradation of GFs, their stimulations on cells are relatively short and discontinuous. In this study, a releasing scaffold platform, consisting of polycaprolactone (PCL) nanofibers and vascular endothelial growth factor (VEGF)-encapsulated gelatin particles, was developed to extend the influence of GFs on mesenchymal stem cells (MSCs) and endothelial cells (ECs). The results showed that this kind of scaffold can direct the differentiation of MSCs to ECs and maintain the stability of the tubular structure, an indicator of the angiogenesis ability of ECs, for an extended period of time. Therefore, the results suggest the potential application of PCL/VEGF-encapsulated gelatin particles (PCL/VGPs) as a growth factor (GF)-releasing scaffold platform in vascular tissue engineering.

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

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

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

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

Johansson, U., et al. (2008). "Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization." Diabetes **57**(9): 2393-2401.

OBJECTIVE: Mesenchymal stem cells (MSCs) contribute to endothelial cell (EC) migration by producing proteases, thereby paving the way into the tissues for ECs. MSCs were added to our previously described composite EC islets as a potential means to improve their capacity for islet angiogenesis. RESEARCH DESIGN AND METHODS: Human islets were coated with primary human bone marrow-derived MSCs and dermal microvascular ECs. The capacity of ECs, with or without MSCs, to adhere to and grow into human islets was analyzed. The survival and functionality of these composite islets were evaluated in a dynamic perifusion assay, and their capacity for angiogenesis in vitro was assessed in a three-dimensional fibrin gel assay. RESULTS: ECs proliferated after culture in MSC-conditioned medium, and MSCs improved the EC coverage threefold compared with EC islets alone. Islet survival in vitro and the functionality of the composite

islets after culture were equal to those of control islets. The EC-MSC islets showed a twofold increase in total sprout formation compared with EC islets, and vascular sprouts emanating from the EC-MSC-islet surface showed migration of ECs into the islets and also into the surrounding matrix, either alone or in concert with MSCs. CONCLUSIONS: EC proliferation, sprout formation, and ingrowth of ECs into the islets were enhanced by MSCs. The use of composite EC-MSC islets may have beneficial effects on revascularization and immune regulation. The technique presented allows for pretreatment of donor islets with recipient-derived ECs and MSCs as a means of improving islet engraftment.

Kamprom, W., et al. (2016). "Effects of mesenchymal stem cell-derived cytokines on the functional properties of endothelial progenitor cells." Eur J Cell Biol **95**(3-5): 153-163.

Human mesenchymal stem cell (hMSC) is a potential source for cell therapy due to its property to promote tissue repair. Although, it has been known that hMSCs promote tissue repair via angiogenic cytokines, the interaction between hMSC-derived cytokines and the endothelial progenitor cells (EPCs), which play an important role in tissue neovascularization, is poorly characterized. We investigate the effect of cytokine released from different sources of hMSCs including bone marrow and gestational tissues on the EPC functions in vitro. The migration, extracellular matrix invasion and vessel formation of EPCs were studied in the presence or absence of cytokines released from various sources of hMSCs using transwell culture system. The migration of EPCs was highest when co-culture with secretory factors from placenta-derived hMSCs (PL-hMSCs) compared to those co-culture with other sources of hMSCs. For invasion and vessel formation, secretory factors from bone marrow-derived hMSCs (BM-hMSCs) could produce the maximal enhancement compared to other sources. We further identified the secreted cytokines and found that the migratory-enhancing cytokine from PL-hMSCs was PDGF-BB while the enhancing cytokine from BM-hMSCs on invasion was IGF-1. For vessel formation, the cytokines released from BM-hMSCs were IGF1 and SDF-1. In conclusion, hMSCs can release angiogenic cytokines which increase the migration, invasion and vessel forming capacity of EPCs. We can then use hMSCs as a source of angiogenic cytokines to induce neovascularization in injured/ischemic tissues.

Kamprom, W., et al. (2016). "Endothelial Progenitor Cell Migration-Enhancing Factors in the Secretome of Placental-Derived Mesenchymal Stem Cells." Stem Cells Int **2016**: 2514326.

Therapeutic potentials of mesenchymal stem cells (MSCs) depend largely on their ability to secrete cytokines or factors that modulate immune response, enhance cell survival, and induce neovascularization in the target tissues. We studied the secretome profile of gestational tissue-derived MSCs and their effects on functions of endothelial progenitor cells (EPCs), another angiogenic cell type that plays an important role during the neovascularization. MSCs derived from placental tissues (PL-MSCs) significantly enhanced EPC migration while BM-MSCs, which are the standard source of MSCs for various clinical applications, did not. By using protein fractionation and mass spectrometry analysis, we identified several novel candidates for EPC migration enhancing factor in PL-MSCs secretome that could be used to enhance neovascularization in the injured/ischemic tissues. We recommend that the strategy developed in our study could be used to systematically identify therapeutically useful molecules in the secretomes of other MSC sources for the clinical applications.

Keats, E. and Z. A. Khan (2012). "Unique responses of stem cell-derived vascular endothelial and mesenchymal cells to high levels of glucose." PLoS One **7**(6): e38752.

Diabetes leads to complications in selected organ systems, and vascular endothelial cell (EC) dysfunction and loss is the key initiating and perpetuating step in the development of these complications. Experimental and clinical studies have shown that hyperglycemia leads to EC dysfunction in diabetes. Vascular stem cells that give rise to endothelial progenitor cells (EPCs) and mesenchymal progenitor cells (MPCs) represent an attractive target for cell therapy for diabetic patients. Whether these vascular stem/progenitor cells succumb to the adverse effects of high glucose remains unknown. We sought to determine whether adult vascular stem/progenitor cells display cellular activation and dysfunction upon exposure to high levels of glucose as seen in

diabetic complications. Mononuclear cell fraction was prepared from adult blood and bone marrow. EPCs and MPCs were derived, characterized, and exposed to either normal glucose (5 mmol/L) or high glucose levels (25 mmol/L). We then assayed for cell activity and molecular changes following both acute and chronic exposure to high glucose. Our results show that high levels of glucose do not alter the derivation of either EPCs or MPCs. The adult blood-derived EPCs were also resistant to the effects of glucose in terms of growth. Acute exposure to high glucose levels increased caspase-3 activity in EPCs (1.4x increase) and mature ECs (2.3x increase). Interestingly, MPCs showed a transient reduction in growth upon glucose challenge. Our results also show that glucose skews the differentiation of MPCs towards the adipocyte lineage while suppressing other mesenchymal lineages. In summary, our studies show that EPCs are resistant to the effects of high levels of glucose, even following chronic exposure. The findings further show that hyperglycemia may have detrimental effects on the MPCs, causing reduced growth and altering the differentiation potential.

Keyhanmanesh, R., et al. (2018). "Systemic Transplantation of Mesenchymal Stem Cells Modulates Endothelial Cell Adhesion Molecules Induced by Ovalbumin in Rat Model of Asthma." Inflammation.

Achieving the optimal clinical outcome of mesenchymal stem cells (MSCs) is particularly dependent on fundamental understanding of therapeutic mechanisms. The current study was focused on the possible mechanisms by which rat bone marrow-derived mesenchymal stem cells (rBMMSCs) and/or conditioned media (CM) display broad immunomodulatory properties for ameliorating of asthma-related pathological changes. Male rats were divided equally into four experimental groups (n = 6): healthy rats received 50 mul PBS intravenously (group C), sensitized rats received 50 mul PBS intravenously (group OVA), sensitized rats received 50 mul CM intravenously (group OVA + CM), and sensitized rats received 50 mul PBS intravenously containing 2 x 10(6) rBMMSCs (group OVA + MSCs). After 2 weeks, the expression of interleukin (IL)-5, IL-12 and INF-gamma, ICAM-1, and VCAM-1; pathological injuries; and the homing of MSCs into the lung tissues were assessed. Our results showed that systemic delivery of rBMMSCs, but not CM, returned the expression of IL-5, IL-12 and INF-gamma, ICAM-1, and VCAM-1 and pathological injuries in the lung tissues of asthmatic groups to the near level of control group ($p < 0.001$ to $p < 0.05$). Moreover, rBMMSCs had potential to successfully recall to asthmatic niche in cell-administrated rats. However, no regulatory function was observed by MSC-CM. Collectively, our data notified the potency of MSCs in ameliorating OVA-mediated airway inflammation in a rat model of asthma presumably by regulating endothelial expression of leukocyte-selective cell adhesion molecules in lung tissue.

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

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

administration of the liver extract in 3D matrigel/collagen culture of HWJMSC/HUVEC can induce hepatocyte marker expression.

Kim, K. C., et al. (2016). "Changes in Caspase-3, B Cell Leukemia/Lymphoma-2, Interleukin-6, Tumor Necrosis Factor-alpha and Vascular Endothelial Growth Factor Gene Expression after Human Umbilical Cord Blood Derived Mesenchymal Stem Cells Transfusion in Pulmonary Hypertension Rat Models." Korean Circ J **46**(1): 79-92.

BACKGROUND AND OBJECTIVES: Failure of vascular smooth muscle apoptosis and inflammatory response in pulmonary arterial hypertension (PAH) is a current research focus. The goals of this study were to determine changes in select gene expressions in monocrotaline (MCT)-induced PAH rat models after human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs) transfusion. MATERIALS AND METHODS: The rats were separated into 3 groups i.e., control group (C group), M group (MCT 60 mg/kg), and U group (hUCB-MSCs transfusion) a week after MCT injection. RESULTS: TUNEL assay showed that the U group had significantly lowered positive apoptotic cells in the lung tissues, as compared with the M group. mRNA of caspase-3, B cell leukemia/lymphoma (Bcl)-2, interleukin (IL)-6, tumor necrosis factor (TNF)-alpha and vascular endothelial growth factor (VEGF) in the lung tissues were greatly reduced at week 4 in the U group. Immunohistochemical staining of the lung tissues also demonstrated a similar pattern, with the exception of IL-6. The protein expression of caspase-3, Bcl-2 VEGF, IL-6, TNF-alpha and brain natriuretic peptide in the heart tissues were significantly lower in the U group, as compared with the M group at week 2. Furthermore, the protein expression of VEGF, IL-6 and BNP in the heart tissues were significantly lower in the U group at week 4. Collagen content in the heart tissues was significantly lower in the U group, as compared with M group at weeks 2 and 4, respectively. CONCLUSION: hUCB-MSCs could prevent inflammation, apoptosis and remodeling in MCT-induced PAH rat models.

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.

Klein, D., et al. (2017). "Mesenchymal Stem Cell Therapy Protects Lungs from Radiation-Induced Endothelial Cell Loss by Restoring Superoxide Dismutase 1 Expression." Antioxid Redox Signal **26**(11): 563-582.

AIMS: Radiation-induced normal tissue toxicity is closely linked to endothelial cell (EC) damage and dysfunction (acute effects). However, the underlying mechanisms of radiation-induced adverse late effects with respect to the vascular compartment remain elusive, and no causative radioprotective treatment is available to date. RESULTS: The importance of injury to EC for radiation-induced late toxicity in lungs after whole

thorax irradiation (WTI) was investigated using a mouse model of radiation-induced pneumopathy. We show that WTI induces EC loss as long-term complication, which is accompanied by the development of fibrosis. Adoptive transfer of mesenchymal stem cells (MSCs) either derived from bone marrow or aorta (vascular wall-resident MSCs) in the early phase after irradiation limited the radiation-induced EC loss and fibrosis progression. Furthermore, MSC-derived culture supernatants rescued the radiation-induced reduction in viability and long-term survival of cultured lung EC. We further identified the antioxidant enzyme superoxide dismutase 1 (SOD1) as a MSC-secreted factor. Importantly, MSC treatment restored the radiation-induced reduction of SOD1 levels after WTI. A similar protective effect was achieved by using the SOD-mimetic EUK134, suggesting that MSC-derived SOD1 is involved in the protective action of MSC, presumably through paracrine signaling. INNOVATION: In this study, we explored the therapeutic potential of MSC therapy to prevent radiation-induced EC loss (late effect) and identified the protective mechanisms of MSC action. CONCLUSIONS: Adoptive transfer of MSCs early after irradiation counteracts radiation-induced vascular damage and EC loss as late adverse effects. The high activity of vascular wall-derived MSCs for radioprotection may be due to their tissue-specific action. Antioxid. Redox Signal. 26, 563-582.

Kokudo, T., et al. (2008). "Snail is required for TGFbeta-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells." J Cell Sci **121**(Pt 20): 3317-3324.

Epithelial-mesenchymal transition (EMT) plays important roles in various physiological and pathological processes, and is regulated by signaling pathways mediated by cytokines, including transforming growth factor beta (TGFbeta). Embryonic endothelial cells also undergo differentiation into mesenchymal cells during heart valve formation and aortic maturation. However, the molecular mechanisms that regulate such endothelial-mesenchymal transition (EndMT) remain to be elucidated. Here we show that TGFbeta plays important roles during mural differentiation of mouse embryonic stem cell-derived endothelial cells (MESECs). TGFbeta2 induced the differentiation of MESECs into mural cells, with a decrease in the expression of the endothelial marker claudin 5, and an increase in expression of the mural markers smooth muscle alpha-actin, SM22alpha and calponin, whereas a TGFbeta type I receptor kinase inhibitor inhibited EndMT. Among the transcription factors involved in EMT, Snail was induced by TGFbeta2 in MESECs. Tetracycline-regulated expression of Snail induced the differentiation of MESECs into mural cells, whereas knockdown of Snail expression abrogated TGFbeta2-induced mural differentiation of MESECs. These results indicate that Snail mediates the actions of endogenous TGFbeta signals that induce EndMT.

Kolbe, M., et al. (2011). "Paracrine effects influenced by cell culture medium and consequences on microvessel-like structures in cocultures of mesenchymal stem cells and outgrowth endothelial cells." Tissue Eng Part A **17**(17-18): 2199-2212.

Mesenchymal stem cells (MSC) from bone marrow and outgrowth endothelial cells (OEC) from peripheral blood are considered as attractive cell types for applications in regenerative medicine aiming to build up complex vascularized tissue-engineered constructs. MSC provide several advantages such as the potential to differentiate to osteoblasts and to support the neovascularization process by release of proangiogenic factors. On the other hand, the neovascularization process can be actively supported by OEC forming perfused vascular structures after co-implantation with other cell types. In this study the formation of angiogenic structures in vitro was investigated in cocultures of MSC and OEC, cultured either in the medium for osteogenic differentiation of MSC (ODM) or in the medium for OEC cultivation endothelial cell growth medium-2 (EGM2 Bullet Kit). After 2 weeks, cocultures in EGM2 formed more microvessel-like structures compared to cocultures in ODM as demonstrated by immunofluorescence staining for the endothelial marker CD31. Increased expression of CD31 and CD146 in quantitative real-time polymerase chain reaction as well as a higher percentage of CD31- and CD146-positive cells in flow cytometry indicated a beneficial influence of EGM2 on endothelial cell growth and function. In addition, the improved formation of vascular structures in EGM2 correlates with

higher levels of the proangiogenic factor vascular endothelial growth factor and platelet-derived growth factor in the supernatant of cocultures as well as in monocultures of MSC when cultivated in EGM-2. Nevertheless, ODM was more suitable for the differentiation of MSC to osteoblastic lineages in the cocultures, whereas EGM2 favored factors involved in vessel stabilization by pericytes. In conclusion, this study highlights the importance of medium components for cell interaction triggering the formation of angiogenic structures.

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.

Kwon, Y. W., et al. (2013). "Tumor necrosis factor-alpha-activated mesenchymal stem cells promote endothelial progenitor cell homing and angiogenesis." Biochim Biophys Acta **1832**(12): 2136-2144.

Mesenchymal stem cells (MSCs) accelerate regeneration of ischemic or injured tissues by stimulation of angiogenesis through a paracrine mechanism. Tumor necrosis factor-alpha (TNF-alpha)-activated MSCs secrete pro-angiogenic cytokines, including IL-6 and IL-8. In the present study, using an ischemic hindlimb animal model, we explored the role of IL-6 and IL-8 in the paracrine stimulation of angiogenesis and tissue regeneration by TNF-alpha-activated MSCs. Intramuscular injection of conditioned medium derived from TNF-alpha-treated MSCs (TNF-alpha CM) into the ischemic hindlimb resulted in attenuated severe limb loss and stimulated blood perfusion and angiogenesis in the ischemic limb. Immunodepletion of IL-6 and IL-8 resulted in attenuated TNF-alpha CM-stimulated tissue repair, blood perfusion, and angiogenesis. In addition, TNF-alpha CM induced migration of human cord blood-derived endothelial progenitor cells (EPCs) through IL-6- and IL-8-dependent mechanisms in vitro. Intramuscular injection of TNF-alpha CM into the ischemic limb led to augmented homing of tail vein-injected EPCs into the ischemic limb in vivo and immunodepletion of IL-6 or IL-8 from TNF-alpha CM attenuated TNF-alpha CM-stimulated homing of EPCs. In addition, intramuscular injection of recombinant IL-6 and IL-8 proteins resulted in increased homing of intravenously transplanted EPCs into the ischemic limb and improved blood perfusion in vivo. These results suggest that TNF-alpha CM stimulates angiogenesis and tissue repair through an increase in homing of EPCs through paracrine mechanisms involving IL-6 and IL-8.

Li, M., et al. (2010). "CXCR4 positive bone mesenchymal stem cells migrate to human endothelial cell stimulated by ox-LDL via SDF-1alpha/CXCR4 signaling axis." Exp Mol Pathol **88**(2): 250-255.

BACKGROUND: Bone mesenchymal stem cells (BMSCs) are attractive candidates for cell based therapies to cardiovascular disease such as infarction and atherosclerosis; however, the mechanisms responsible for stem cell chemotaxis and homing remain unknown. Chemokine stromal cell-derived factor 1 (SDF-1alpha) is involved in the process of atherogenesis. This study was aimed at investigating whether the SDF-1alpha of human umbilical vein endothelial cells (HUVECs) plays a role in migration of BM-derived CXCR4(+)(receptor for SDF-1alpha) stem cells. METHODS: HUVECs were cultured from human umbilical cords and was treated with ox-LDL. The mRNA and protein

expression of SDF-1alpha was detected in HUVECs. CXCR4(+)BMSCs from bone marrow were isolated and were tested by migration and adhesion assays. RESULTS: It was found that ox-LDL induced HUVECs to increase the mRNA and protein expression of SDF-1alpha. Ox-LDL increased the migratory and adhesion response of CXCR4(+)BMSCs. When the neutralizing SDF-1alpha antibody abrogated the secreted SDF-1alpha, the migration and adhesion response of CXCR4(+)BMSCs markedly decreased. CONCLUSIONS: Our data indicated that the endothelial cells (ECs) stimulated by ox-LDL could increase the BMSCs migratory response via SDF-1alpha/CXCR4 signaling axis. These findings provide a new paradigm for biological effects of ox-LDL and have implications for novel stem cell therapeutic strategies for atherosclerosis.

Li, Q., et al. (2017). "VEGF treatment promotes bone marrow-derived CXCR4(+) mesenchymal stromal stem cell differentiation into vessel endothelial cells." Exp Ther Med **13**(2): 449-454.

Stem/progenitor cells serve an important role in the process of blood vessel repair. However, the mechanism of vascular repair mediated by C-X-C chemokine receptor type 4-positive (CXCR4(+)) bone marrow-derived mesenchymal stem cells (BMSCs) following myocardial infarction remains unclear. The aim of the present study was to investigate the effects of vascular endothelial growth factor (VEGF) on vessel endothelial differentiation from BMSCs. CXCR4(+) BMSCs were isolated from the femoral bone marrow of 2-month-old mice and the cells were treated with VEGF. Expression of endothelial cell markers and the functional properties were assessed by reverse transcription-quantitative polymerase chain reaction, flow cytometry and vascular formation analyses. The results indicated that the CXCR4(+) BMSCs from femoral bone marrow cells expressed putative cell surface markers of mesenchymal stem cells. Treatment with VEGF induced platelet/endothelial cell adhesion molecule-1 (PECAM-1) and von Willebrand factor (vWF) expression at the transcriptional and translational levels, compared with untreated controls. Moreover, VEGF treatment induced CXCR4(+) BMSCs to form hollow tube-like structures on Matrigel, suggesting that the differentiated endothelial cells had the functional properties of blood vessels. The results demonstrate that the CXCR4(+) BMSCs were able to differentiate into vessel endothelial cells following VEGF treatment. For cell transplantation in vascular disease, it may be concluded that CXCR4(+) BMSCs are a novel source of endothelial progenitor cells with high potential for application in vascular repair.

Li, Q., et al. (2007). "[Investigation of canine mesenchymal stem cells differentiation to vascular endothelial cell in vitro]." Sheng Wu Yi Xue Gong Cheng Xue Za Zhi **24**(6): 1348-1351.

To induce endothelial cell, canine bone marrow-derived mesenchymal stem cells (MSCs) were separated from bone marrow by density gradient centrifugation. The isolated MSCs were induced to form endothelial-like cell in the presence of vascular endothelial growth factor (VEGF), endothelial growth factor (EGF) and so on. These results showed that the cells uniformly took on a cobblestone morphology under the light microscope, and cell nucleolus was in the middle of the cells. The cells displayed Weibel-Palade bodies under the transmission electron microscope. vWF, a specific marker of endothelial cell was positive in the cells. The above results demonstrate that MSCs may be differentiated into endothelial cells in vitro.

Li, Y., et al. (2018). "Evidence for Kaposi Sarcoma Originating from Mesenchymal Stem Cell through KSHV-induced Mesenchymal-to-Endothelial Transition." Cancer Res **78**(1): 230-245.

The major transmission route for Kaposi sarcoma-associated herpesvirus (KSHV) infection is the oral cavity through saliva. Kaposi sarcoma (KS) frequently occurs in the oral cavity in HIV-positive individuals and is often the first presenting sign of AIDS. However, the oral target cells for KSHV infection and the cellular origin of Kaposi sarcoma remain unknown. Here we present clinical and experimental evidences that Kaposi sarcoma spindle cells may originate from virally modified oral mesenchymal stem cells (MSC). AIDS-KS spindle cells expressed neuroectodermal stem cell marker (Nestin) and oral MSC marker CD29, suggesting an oral/craniofacial MSC lineage of AIDS-associated Kaposi sarcoma. Furthermore, oral MSCs were highly susceptible to KSHV infection, and infection promoted multilineage differentiation and mesenchymal-to-endothelial transition (MEndT). KSHV

infection of oral MSCs resulted in expression of a large number of cytokines, a characteristic of Kaposi sarcoma, and upregulation of Kaposi sarcoma signature and MEndT-associated genes. These results suggest that Kaposi sarcoma may originate from pluripotent MSC and KSHV infection transforms MSC to Kaposi sarcoma-like cells through MEndT.Significance: These findings indicate that Kaposi sarcomas, which arise frequently in AIDS patients, originate from neural crest-derived mesenchymal stem cells, with possible implications for improving the clnical treatment of this malignancy. Cancer Res; 78(1); 230-45. (c)2017 AACR.

Loibl, M., et al. (2014). "Direct cell-cell contact between mesenchymal stem cells and endothelial progenitor cells induces a pericyte-like phenotype in vitro." Biomed Res Int **2014**: 395781.

Tissue engineering techniques for the regeneration of large bone defects require sufficient vascularisation of the applied constructs to ensure a sufficient supply of oxygen and nutrients. In our previous work, prevascularised 3D scaffolds have been successfully established by coculture of bone marrow derived stem cells (MSCs) and endothelial progenitor cells (EPCs). We identified stabilising pericytes (PCs) as part of newly formed capillary-like structures. In the present study, we report preliminary data on the interactions between MSCs and EPCs, leading to the differentiation of pericyte-like cells. MSCs and EPCs were seeded in transwell cultures, direct cocultures, and single cultures. Cells were cultured for 10 days in IMDM 10% FCS or IMDM 5% FCS 5% platelet lysate medium. Gene expression of PC markers, CD146, NG2, alphaSMA, and PDGFR-beta, was analysed using RT-PCR at days 0, 3, 7, and 10. The upregulation of CD146, NG2, and alphaSMA in MSCs in direct coculture with EPCs advocates the MSCs' differentiation towards a pericyte-like phenotype in vitro. These results suggest that pericyte-like cells derive from MSCs and that cell-cell contact with EPCs is an important factor for this differentiation process. These findings emphasise the concept of coculture strategies to promote angiogenesis for cell-based tissue engineered bone grafts.

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

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

Lozito, T. P., et al. (2009). "Mesenchymal stem cell modification of endothelial matrix regulates their vascular differentiation." J Cell Biochem **107**(4): 706-713.

Mesenchymal stem cells (MSCs) respond to a variety of differentiation signal provided by their local environments. A large portion of these signals originate from the extracellular matrix (ECM). At the same time, MSCs secrete various matrix-altering agents, including proteases, that alter ECM-encoded differentiation signals. Here we investigated the interactions between MSC and ECM produced by endothelial cells (EC-matrix), focusing not only on the differentiation signals provided by EC-matrix, but also on MSC-alteration of these signals and the resultant affects on MSC differentiation. MSCs were

cultured on EC-matrix modified in one of three distinct ways. First, MSCs cultured on native EC-matrix underwent endothelial cell (EC) differentiation early during the culture period and smooth muscle cell (SMC) differentiation at later time points. Second, MSCs cultured on crosslinked EC-matrix, which is resistant to MSC modification, differentiated towards an EC lineage only. Third, MSCs cultured on EC-matrix pre-modified by MSCs underwent SMC-differentiation only. These MSC-induced matrix alterations were found to deplete the factors responsible for EC-differentiation, yet activate the SMC-differentiation factors. In conclusion, our results demonstrate that the EC-matrix contains factors that support MSC differentiation into both ECs and SMCs, and that these factors are modified by MSC-secreted agents. By analyzing the framework by which EC-matrix regulates differentiation in MSCs, we have uncovered evidence of a feedback system in which MSCs are able to alter the very matrix signals acting upon them.

Luo, Y., et al. (2012). "Pretreating mesenchymal stem cells with interleukin-1beta and transforming growth factor-beta synergistically increases vascular endothelial growth factor production and improves mesenchymal stem cell-mediated myocardial protection after acute ischemia." Surgery **151**(3): 353-363.

BACKGROUND: Mesenchymal stem cells (MSCs) improve postischemic myocardial function in part through their secretion of growth factors such as vascular endothelial growth factor (VEGF). Pretreating MSCs with various cytokines or small molecules can improve VEGF secretion and MSC-mediated cardioprotection. However, whether 1 cytokine can potentiate the effect of another cytokine in MSC pretreatment to achieve a synergistic effect on VEGF production and cardioprotection is poorly studied. METHODS: MSCs were treated with interleukin (IL)-1beta and/or transforming growth factor (TGF)-beta1 for 24 hours before experiments. VEGF production was determined by enzyme-linked immunosorbent assay. Isolated hearts from adult male Sprague-Dawley rats were subjected to 15 minutes of equilibration, 25 minutes of ischemia, and 40 minutes reperfusion. Hearts (n = 5-7 per group) were randomly infused with vehicle, untreated MSCs, or MSCs pretreated with IL-1beta and/or TGF-beta1. Specific inhibitors were used to delineate the roles of p38 mitogen-activated protein kinase (MAPK) and SMAD3 in IL-1beta- and TGF-beta1-mediated stimulation of MSCs. RESULTS: MSCs cotreated with IL-1beta and TGF-beta1 exhibited synergistically increased VEGF secretion, and they greatly improved postischemic myocardial functional recovery. Ablation of p38 MAPK and SMAD3 activation with specific inhibitors negated both IL-1beta- and TGF-beta1-mediated VEGF production in MSCs and the ability of these pretreated MSCs to improve myocardial recovery after ischemia. CONCLUSION: Pretreating MSCs with 2 cytokines may be useful to fully realize the potential of cell-based therapies for ischemic tissues.

Matsumoto, R., et al. (2005). "Vascular endothelial growth factor-expressing mesenchymal stem cell transplantation for the treatment of acute myocardial infarction." Arterioscler Thromb Vasc Biol **25**(6): 1168-1173.

OBJECTIVE: Vascular endothelial growth factor (VEGF) plays an important role in inducing angiogenesis. Mesenchymal stem cells (MSCs) may have potential for differentiation to several types of cells, including myocytes. We hypothesized that transplantation of VEGF-expressing MSCs could effectively treat acute myocardial infarction (MI) by providing enhanced cardioprotection, followed by angiogenic effects in salvaging ischemic myocardium. METHODS AND RESULTS: The human VEGF165 gene was transfected to cultured MSCs of Lewis rats using an adenoviral vector. Six million VEGF-transfected and LacZ-transfected MSCs (VEGF group), LacZ-transfected MSCs (control group), or serum-free medium only (medium group) were injected into syngeneic rat hearts 1 hour after left coronary artery occlusion. At 1 week after MI, MSCs were detected by X-gal staining in infarcted region. High expression of VEGF was immunostained in the VEGF group. At 28 days after MI, infarct size, left ventricular dimensions, ejection fraction, E wave/A wave ratio and capillary density of the infarcted region were most improved in the VEGF group, compared with the medium group. Immunohistochemically, alpha-smooth muscle actin-positive cells were most increased in the VEGF group. CONCLUSIONS: This combined strategy of

cell transplantation with gene therapy could be a useful therapy for the treatment of acute MI.

Matsushita, T., et al. (2011). "Mesenchymal stem cells transmigrate across brain microvascular endothelial cell monolayers through transiently formed inter-endothelial gaps." Neurosci Lett **502**(1): 41-45.

Mesenchymal stem cells (MSCs) hold much promise for cell therapy for neurological diseases such as cerebral ischemia and Parkinson's disease. Intravenously administered MSCs accumulate in lesions within the brain parenchyma, but little is known of the details of MSC transmigration across the blood-brain barrier (BBB). To study MSC transmigration across the BBB, we developed an in vitro culture system consisting of rat brain microvascular endothelial cells (BMECs) and bone marrow-derived MSCs using Transwell or Millicell culture inserts. Using this system, we first investigated the influence of the number of MSCs added to the upper chamber on BMEC barrier integrity. The addition of MSCs at a density of 1.5 x 10(5) cells/cm(2) led to disruption of the BMEC monolayer structure and decreased barrier function as measured by the transendothelial electrical resistance (TEER). When applied at a density of 1.5 x 10(4) cells/cm(2), neither remarkable disruption of the BMEC monolayers nor a significant decrease in TEER was observed until at least 12 h. After cultivation for 24 h under this condition, MSCs were found in the subendothelial space or beneath the insert membrane, suggesting that MSCs transmigrate across BMEC monolayers. Time-lapse imaging revealed that MSCs transmigrated across the BMEC monolayers through transiently formed intercellular gaps between the BMECs. These results show that our in vitro culture system consisting of BMECs and MSCs is useful for investigating the molecular and cellular mechanisms underlying MSC transmigration across the BBB.

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

This paper demonstrates a method to engineer, in vitro, a nascent microvasculature within a collagen-glycosaminoglycan scaffold with a view to overcoming the major issue of graft failure due to avascular necrosis of tissue-engineered constructs. Human umbilical vein endothelial cells (ECs) were cultured alone and in various co-culture combinations with human mesenchymal stem cells (MSCs) to determine their vasculogenic abilities in vitro. Results demonstrated that the delayed addition of MSCs to pre-formed EC networks, whereby MSCs act as pericytes to the nascent vessels, resulted in the best developed vasculature. The results also demonstrate that the crosstalk between ECs and MSCs during microvessel formation occurs in a highly regulated, spatio-temporal fashion, whereby the initial seeding of ECs results in platelet derived growth factor (PDGF) release; the subsequent addition of MSCs 3 days later leads to a cessation in PDGF production, coinciding with increased vascular endothelial cell growth factor expression and enhanced vessel formation. Functional assessment of these pre-engineered constructs in a subcutaneous rat implant model demonstrated anastomosis between the in vitro engineered vessels and the host vasculature, with significantly increased vascularization occurring in the co-culture group. This study has thus provided new information on the process of in vitro vasculogenesis within a three-dimensional porous scaffold for tissue engineering and demonstrates the potential for using these vascularized scaffolds in the repair of critical sized bone defects.

Medici, D. and R. Kalluri (2012). "Endothelial-mesenchymal transition and its contribution to the emergence of stem cell phenotype." Semin Cancer Biol **22**(5-6): 379-384.

Vascular endothelial cells can demonstrate considerable plasticity to generate other cell types during embryonic development and disease progression. This process occurs through a cell differentiation mechanism known as endothelial-mesenchymal transition (EndMT). The generation of mesenchymal cells from endothelium is a crucial step in endothelial cell differentiation to several lineages including fibroblasts, myofibroblasts, mural cells, osteoblasts, chondrocytes, and adipocytes. Such differentiation patterns have been observed in systems of cardiac development, fibrosis, diabetic nephropathy, heterotopic ossification and cancer. Here we describe the EndMT program and discuss the current evidence of EndMT-mediated

acquisition of stem cell characteristics and multipotent differentiation capabilities.

Meng, S. S., et al. (2018). "mTOR/STAT-3 pathway mediates mesenchymal stem cell-secreted hepatocyte growth factor protective effects against lipopolysaccharide-induced vascular endothelial barrier dysfunction and apoptosis." J Cell Biochem.

Mesenchymal stem cells (MSCs) protect the endothelial barrier complex and survival, implicated in the pathogenesis of acute lung injury (ALI) via paracrine hepatocyte growth factor (HGF). However, the mechanism of HGF in endothelial regulation remains unclear. Here, we introduced a coculture protocol of pulmonary microvascular endothelial cells (PMVECs) and overexpression of the HGF gene of MSCs (MSC-HGF). Immunofluorescence and endothelial permeability analysis revealed that MSC-HGF protected endothelial tight junction protein occludin expression and attenuated cellular permeability as well as endothelial apoptosis. To investigate the novel mechanism mammalian TOR (mTOR)/ signal transducer and activator of transcription 3 (STAT-3) signaling in HGF protective effects against endothelial barrier and apoptosis, we used recombinant mouse HGF in endothelial cells. In addition, we used mTOR inhibitor rapamycin to inhibit the mTOR pathway. Our study demonstrated that rapamycin decreased the protective effects of HGF on the endothelium by decreasing tight junction protein occludin expression and cell proliferation, and raising lipopolysaccharide (LPS)-induced endothelial permeability, endothelial cell injury factors ET-1 and vWF. Similarly, the protective effects of HGF on reducing endothelial barrier and apoptosis were weakened when PMVECs were treated with the STAT-3 inhibitor S3I-201. Moreover, mTOR/STAT-3 were activated by HGF demonstrated as raising mTOR (Ser2448) and STAT3 (Ser727) phosphorylation proteins, leading to endothelial barrier improvement and survival. Reversely, rapamycin or S3I-201 inhibited mTOR/STAT-3 activation. Taken together, our findings highlight that the activation of the mTOR/STAT-3 pathway provides novel mechanistic insights into MSC-secreted HGF protection against LPS-induced vascular endothelial permeability dysfunction and apoptosis, which contributes to decreasing microvascular loss and lung injury.

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

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

Mikami, S., et al. (2013). "Autologous bone-marrow mesenchymal stem cell implantation and endothelial function in a rabbit ischemic limb model." PLoS One **8**(7): e67739.

BACKGROUND: The purpose of this study was to determine whether autologous mesenchymal stem cells (MSCs) implantation improves endothelial dysfunction in a rabbit ischemic limb model. METHODS: We evaluated the effect of MSC implantation on limb blood flow (LBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP), an endothelium-independent vasodilator, in rabbits with limb ischemia in which cultured MSCs were

implanted (n = 20) or saline was injected as a control group (n = 20). LBF was measured using an electromagnetic flowmeter. A total of 10(6) MSCs were implanted into each ischemic limb. RESULTS: Histological sections of ischemic muscle showed that capillary index (capillary/muscle fiber) was greater in the MSC implantation group than in the control group. Laser Doppler blood perfusion index was significantly increased in the MSC implantation group compared with that in the control group. LBF response to ACh was greater in the MSC group than in the control group. After administration of N(G)-nitro-L-arginine, a nitric oxide synthase inhibitor, LBF response to ACh was similar in the MSC implantation group and control group. Vasodilatory effects of SNP in the two groups were similar. CONCLUSIONS: These findings suggest that MSC implantation induces angiogenesis and augments endothelium-dependent vasodilation in a rabbit ischemic model through an increase in nitric oxide production.

Nakahara, M., et al. (2013). "Corneal endothelial expansion promoted by human bone marrow mesenchymal stem cell-derived conditioned medium." PLoS One **8**(7): e69009.

Healthy corneal endothelium is essential for maintaining corneal clarity, as the damage of corneal endothelial cells and loss of cell count causes severe visual impairment. Corneal transplantation is currently the only therapy for severe corneal disorders. The greatly limited proliferative ability of human corneal endothelial cells (HCECs), even in vitro, has challenged researchers to establish efficient techniques for the cultivating HCECs, a pivotal issue for clinical applications. The aim of this study was to evaluate conditioned medium (CM) obtained from human bone marrow-derived mesenchymal stem cells (MSCs) (MSC-CM) for use as a consistent expansion protocol of HCECs. When HCECs were maintained in the presence of MSC-CM, cell morphology assumed a hexagonal shape similar to corneal endothelial cells in vivo, as opposed to the irregular cell shape observed in control cultures in the absence of MSC-CM. They also maintained the functional protein phenotypes; ZO-1 and Na(+)/K(+)-ATPase were localized at the intercellular adherent junctions and pump proteins of corneal endothelium were accordingly expressed. In comparison to the proliferative potential observed in the control cultures, HCECs maintained in MSC-CM were found to have more than twice as many Ki67-positive cells and a greatly increased incorporation of BrdU into DNA. MSC-CM further facilitated the cell migration of HCECs. Lastly, the mechanism of cell proliferation mediated by MSC-CM was investigated, and phosphorylation of Akt and ERK1/2 was observed in HCECs after exposure to MSC-CM. The inhibitor to PI 3-kinase maintained the level of p27(Kip1) for up to 24 hours and greatly blocked the expression of cyclin D1 and D3 during the early G1 phase, leading to the reduction of cell density. These findings indicate that MSC-CM not only stimulates the proliferation of HCECs by regulating the G1 proteins of the cell cycle but also maintains the characteristic differentiated phenotypes necessary for the endothelial functions.

Nguyen, B. B., et al. (2017). "Collagen hydrogel scaffold promotes mesenchymal stem cell and endothelial cell coculture for bone tissue engineering." J Biomed Mater Res A **105**(4): 1123-1131.

The generation of functional, vascularized tissues is a key challenge for the field of tissue engineering. Before clinical implantations of such tissue engineered bone constructs can succeed, tactics to promote neovascularization need to be strengthened. We have previously demonstrated that the tubular perfusion system (TPS) bioreactor is an effective culturing method to augment osteogenic differentiation and maintain viability of human mesenchymal stem cells (hMSC). Here, we devised a strategy to address the need for a functional microvasculature by designing an in vitro coculture system that simultaneously cultures osteogenic differentiating hMSCs with endothelial cells (ECs). We utilized the TPS bioreactor as a dynamic coculture environment, which we hypothesize will encourage prevascularization of endothelial cells and early formation of bone tissue and could aid in anastomosis of the graft with the host vasculature after patient implantation. To evaluate the effect of different natural scaffolds for this coculture system, the cells were encapsulated in alginate and/or collagen hydrogel scaffolds. We discovered the necessity of cell-to-cell proximity between the two cell types as well as preference for the natural cell binding capabilities of hydrogels like collagen. We discovered increased osteogenic and angiogenic potential as seen by

amplified gene and protein expression of ALP, BMP-2, VEGF, and PECAM. The TPS bioreactor further augmented these expressions, indicating a synergistic effect between coculture and applied shear stress. The development of this dynamic coculture platform for the prevascularization of engineered bone, emphasizing the importance of the construct microenvironments and will advance the clinical use of tissue engineered constructs. (c) 2017 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 105A: 1123-1131, 2017.

Ning, H., et al. (2011). "Mesenchymal stem cell marker Stro-1 is a 75 kd endothelial antigen." Biochem Biophys Res Commun **413**(2): 353-357.

Stro-1 is the best-known mesenchymal stem cell (MSC) marker. However, previous studies have observed its expression in the endothelium. In the present study we performed immunofluorescence (IF) staining for Stro-1, using endothelial marker vWF as reference. In the liver, both proteins were expressed in the endothelium of the central veins and hepatic sinusoids. In the lung, both were expressed in the endothelium of pulmonary blood vessels, but while vWF was absent in the alveolar capillaries, Stro-1 was present. In the kidney, both were expressed in the endothelium of renal arterial branches, but while vWF was strongly expressed in the glomeruli, Stro-1 only scantly. IF staining in cultured endothelial cells also showed extensive overlaps between Stro-1 and vWF. Western blot analysis with Stro-1 antibody detected a single protein band of 75 kd in endothelial cells but not smooth muscle cells, fibroblasts, or B cells. Cancer cell lines PC3, DU145, MCF7, and K562 were also positive. Adipose-derived stem cells (ADSCs) expressed higher levels of Stro-1 when cultured beyond the first passage or when induced to differentiate into endothelial cells. These data, together with previous studies, indicate that Stro-1 is intrinsically an endothelial antigen, and its expression in MSC is probably an induced event.

Park, Y. S., et al. (2015). "CCN1 secreted by tonsil-derived mesenchymal stem cells promotes endothelial cell angiogenesis via integrin alphav beta3 and AMPK." J Cell Physiol **230**(1): 140-149.

CCN1 is highly expressed in cancer cells and has been identified in the secretome of bone marrow-derived mesenchymal stem cells (BM-MSC). Although secreted CCN1 is known to promote angiogenesis, its underlying mechanism remains unclear. Here, we examined whether our recently-established tonsil-derived MSC (T-MSC) secrete CCN1 and, if any, how CCN1 promotes the angiogenesis of human umbilical vein endothelial cells (HUVEC). Compared with untreated control T-MSC, a higher level of CCN1 was secreted by T-MSC treated with activin A and sonic hedgehog, drugs known to induce endodermal differentiation. Expectedly, conditioned medium collected from differentiated T-MSC (DCM) significantly increased HUVEC migration and tube formation compared with that from control T-MSC (CCM), and these stimulatory effects were reversed by neutralization with anti-CCN1 antibody. Treatment with recombinant human CCN1 (rh-CCN1) alone also mimicked the stimulatory effects of DCM. Furthermore, treatment with either DCM or rh-CCN1 increased the phosphorylation of AMP kinase (AMPK), and ectopic expression of siRNA of the AMPK gene inhibited all observed effects of both DCM and rh-CCN1. However, no alteration of intracellular ATP levels or phosphorylation of LKB1, a well-known upstream factor of AMPK activation, was observed under our conditions. Finally, the neutralization of integrin alpha(v) beta(3) with anti-integrin alpha(v) beta(3) antibody almost completely reversed the effects of CCN1 on AMPK phosphorylation, and EC migration and tube formation. Taken together, we demonstrated that T-MSC increase the secretion of CCN1 in response to endodermal differentiation and that integrin alpha(v) beta(3) and AMPK mediate CCN1-induced EC migration and tube formation independent of intracellular ATP levels alteration.

Pedersen, T. O., et al. (2012). "Osteogenic stimulatory conditions enhance growth and maturation of endothelial cell microvascular networks in culture with mesenchymal stem cells." J Tissue Eng **3**(1): 2041731412443236.

To optimize culture conditions for in vitro prevascularization of tissue-engineered bone constructs, the development of organotypic blood vessels under osteogenic stimulatory conditions (OM) was investigated. Coculture of endothelial cells and mesenchymal stem cells was used to assess proangiogenic effects of mesenchymal stem cells on endothelial cells. Four different culture conditions were

evaluated for their effect on development of microvascular endothelial cell networks. Mineralization, deposition of extracellular matrix, and perivascular gene expression were studied in OM. After 3 days, endothelial cells established elongated capillary-like networks, and upregulated expression of vascular markers was seen. After 15 days, all parameters evaluated were significantly increased for cultures in OM. Mature networks developed in OM presented lumens enveloped by basement membrane-like collagen IV, with obvious mineralization and upregulated perivascular gene expression from mesenchymal stem cells. Our results suggest osteogenic stimulatory conditions to be appropriate for in vitro development of vascularized bone implants for tissue engineering.

Pedersen, T. O., et al. (2014). "Mesenchymal stem cells induce endothelial cell quiescence and promote capillary formation." Stem Cell Res Ther **5**(1): 23.

INTRODUCTION: Rapid establishment of functional blood vessels is a prerequisite for successful tissue engineering. During vascular development, endothelial cells (ECs) and perivascular cells assemble into a complex regulating proliferation of ECs, vessel diameter and production of extracellular matrix proteins. The aim of this study was to evaluate the ability of mesenchymal stem cells (MSCs) to establish an endothelial-perivascular complex in tissue-engineered constructs comprising ECs and MSCs. METHODS: Primary human ECs and MSCs were seeded onto poly(L-lactide-co-1,5-dioxepan-2-one) (poly(LLA-co-DXO)) scaffolds and grown in dynamic culture before subcutaneous implantation in immunocompromised mice for 1 and 3 weeks. Cellular activity, angiogenic stimulation and vascular assembly in cell/scaffold constructs seeded with ECs or ECs/MSCs in a 5:1 ratio was monitored with real-time RT-PCR, ELISA and immunohistochemical microscopy analysis. RESULTS: A quiescent phenotype of ECs was generated, by adding MSCs to the culture system. Decreased proliferation of ECs, in addition to up-regulation of selected markers for vascular maturation was demonstrated. Baseline expression of VEGFa was higher for MSCs compared with EC ($P<0.001$), with subsequent up-regulated VEGFa-expression for EC/MSC constructs before ($P<0.05$) and after implantation ($P<0.01$). Furthermore, an inflammatory response with CD11b+cells was generated from implantation of human cells. At the end of the 3 week experimental period, a higher vascular density was shown for both cellular constructs compared with empty control scaffolds ($P<0.01$), with the highest density of capillaries being generated in constructs comprising both ECs and MSCs. CONCLUSIONS: Induction of a quiescent phenotype of ECs associated with vascular maturation can be achieved by co-seeding with MSCs. Hence, MSCs can be appropriate perivascular cells for tissue-engineered constructs.

Penarando, J., et al. (2018). "A role for endothelial nitric oxide synthase in intestinal stem cell proliferation and mesenchymal colorectal cancer." BMC Biol **16**(1): 3.

BACKGROUND: Nitric oxide (NO) has been highlighted as an important agent in cancer-related events. Although the inducible nitric oxide synthase (iNOS) isoform has received most attention, recent studies in the literature indicate that the endothelial isoenzyme (eNOS) can also modulate different tumor processes including resistance, angiogenesis, invasion, and metastasis. However, the role of eNOS in cancer stem cell (CSC) biology and mesenchymal tumors is unknown. RESULTS: Here, we show that eNOS was significantly upregulated in VilCre (ERT2) Apc (fl/+) and VilCre (ERT2) Apc (fl/fl) mouse intestinal tissue, with intense immunostaining in hyperproliferative crypts. Similarly, the more invasive VilCre (ERT2) Apc (fl/+) Pten (fl/+) mouse model showed an overexpression of eNOS in intestinal tumors whereas this isoform was not expressed in normal tissue. However, none of the three models showed iNOS expression. Notably, when 40 human colorectal tumors were classified into different clinically relevant molecular subtypes, high eNOS expression was found in the poor relapse-free and overall survival mesenchymal subtype, whereas iNOS was absent. Furthermore, Apc (fl/fl) organoids overexpressed eNOS compared with wild-type organoids and NO depletion with the scavenger carboxy-PTIO (c-PTIO) decreased the proliferation and the expression of stem-cell markers, such as Lgr5, Troy, Vav3, and Slc14a1, in these intestinal organoids. Moreover, specific NO depletion also decreased the expression of CSC-related

proteins in human colorectal cancer cells such as beta-catenin and Bmi1, impairing the CSC phenotype. To rule out the contribution of iNOS in this effect, we established an iNOS-knockdown colorectal cancer cell line. NO-depleted cells showed a decreased capacity to form tumors and c-PTIO treatment in vivo showed an antitumoral effect in a xenograft mouse model. CONCLUSION: Our data support that eNOS upregulation occurs after Apc loss, emerging as an unexpected potential new target in poor-prognosis mesenchymal colorectal tumors, where NO scavenging could represent an interesting therapeutic alternative to targeting the CSC subpopulation.

Peters, E. B., et al. (2015). "CD45+ Cells Present Within Mesenchymal Stem Cell Populations Affect Network Formation of Blood-Derived Endothelial Outgrowth Cells." Biores Open Access **4**(1): 75-88.

Mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs) represent promising cell sources for angiogenic therapies. There are, however, conflicting reports regarding the ability of MSCs to support network formation of endothelial cells. The goal of this study was to assess the ability of human bone marrow-derived MSCs to support network formation of endothelial outgrowth cells (EOCs) derived from umbilical cord blood EPCs. We hypothesized that upon in vitro coculture, MSCs and EOCs promote a microenvironment conducive for EOC network formation without the addition of angiogenic growth supplements. EOC networks formed by coculture with MSCs underwent regression and cell loss by day 10 with a near 4-fold and 2-fold reduction in branch points and mean segment length, respectively, in comparison with networks formed by coculture vascular smooth muscle cell (SMC) cocultures. EOC network regression in MSC cocultures was not caused by lack of vascular endothelial growth factor (VEGF)-A or changes in TGF-beta1 or Ang-2 supernatant concentrations in comparison with SMC cocultures. Removal of CD45+ cells from MSCs improved EOC network formation through a 2-fold increase in total segment length and number of branch points in comparison to unsorted MSCs by day 6. These improvements, however, were not sustained by day 10. CD45 expression in MSC cocultures correlated with EOC network regression with a 5-fold increase between day 6 and day 10 of culture. The addition of supplemental growth factors VEGF, fibroblastic growth factor-2, EGF, hydrocortisone, insulin growth factor-1, ascorbic acid, and heparin to MSC cocultures promoted stable EOC network formation over 2 weeks in vitro, without affecting CD45 expression, as evidenced by a lack of significant differences in total segment length (p=0.96). These findings demonstrate the ability of MSCs to support EOC network formation correlates with removal of CD45+ cells and improves upon the addition of soluble growth factors.

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

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

gene or cell surface expression of E-selectin, P-selectin, VCAM1 and ICAM1. Inhibition of p38 MAPK, but not ERK-1,2, in endothelial cells completely abrogated the stimulation of the mesenchymal stem cell adhesion by von Willebrand factor. CONCLUSIONS: Von Willebrand factor is an auto/paracrine regulator of endothelial cells. Activation of p38 MAPK in endothelial cells by von Willebrand factor is responsible for the regulation of endothelial cell adhesiveness for mesenchymal stem cells.

Potapova, I. A., et al. (2013). "Caspases and p38 MAPK regulate endothelial cell adhesiveness for mesenchymal stem cells." PLoS One **8**(9): e73929.

Mesenchymal stem cells natively circulating or delivered into the blood stream home to sites of injury. The mechanism of mesenchymal stem cell homing to sites of injury is poorly understood. We have shown that the development of apoptosis in endothelial cells stimulates endothelial cell adhesiveness for mesenchymal stem cells. Adhesion of mesenchymal stem cells to apoptotic endothelial cells depends on the activation of endothelial caspases and p38 MAPK. Activation of p38 MAPK in endothelial cells has a primary effect while the activation of caspases potentiates the mesenchymal stem cell adhesion. Overall, our study of the mesenchymal stem cell interaction with endothelial cells indicates that mesenchymal stem cells recognize and specifically adhere to distressed/apoptotic endothelial cells.

Qiu, X., et al. (2012). "Combined strategy of mesenchymal stem cell injection with vascular endothelial growth factor gene therapy for the treatment of diabetes-associated erectile dysfunction." J Androl **33**(1): 37-44.

This study was designed to investigate the effect of vascular endothelial growth factor 164 adenovirus (Ad-VEGF(164))-transfected mesenchymal stem cells (MSC) on improving erectile function in diabetic rats. Forty-five male Sprague-Dawley rats were injected with streptozotocin to develop type 1 diabetes, whereas 10 served as normal controls. Diabetic rats were randomly divided into 3 groups: rats that underwent intracavernous injection with phosphate-buffered saline (DM+PBS), unmodified MSCs (DM+MSC), and Ad-VEGF(164)-transfected MSCs (DM+VMSC). Normal controls received intracavernous injection of PBS. Four weeks after injection, erectile function was measured by cavernous nerve electrostimulation. Penile tissue was harvested for histology and enzyme-linked immunoassay. Prior to injection, high expression of VEGF was confirmed in Ad-VEGF(164)-transfected MSCs by enzyme-linked immunoassay. Four weeks after injection, the erectile function, as well as the content of smooth muscle and endothelium in corpus cavernosum increased significantly in the MSC-injected groups compared with the DM+PBS group. There was a significant improvement of erectile function, the content of smooth muscle and endothelium, and the VEGF concentration in the corpus cavernosum in the DM+VMSC group compared with the DM+MSC group. Our study validates the effect of intracavernous injection of MSCs for diabetes-associated erectile dysfunction in an animal model. The combined strategy of MSC injection with VEGF gene therapy-enhanced therapy of MSCs for the treatment of diabetes-associated erectile dysfunction.

Robinson, S. T., et al. (2016). "A novel platelet lysate hydrogel for endothelial cell and mesenchymal stem cell-directed neovascularization." Acta Biomater **36**: 86-98.

UNLABELLED: Mesenchymal stem cells (MSC) hold promise in promoting vascular regeneration of ischemic tissue in conditions like critical limb ischemia of the leg. However, this approach has been limited in part by poor cell retention and survival after delivery. New biomaterials offer an opportunity to localize cells to the desired tissue after delivery, but also to improve cell survival after delivery. Here we characterize the mechanical and microstructural properties of a novel hydrogel composed of pooled human platelet lysate (PL) and test its ability to promote MSC angiogenic activity using clinically relevant in vitro and in vivo models. This PL hydrogel had comparable storage and loss modulus and behaved as a viscoelastic solid similar to fibrin hydrogels despite having 1/4-1/10th the fibrin content of standard fibrin gels. Additionally, PL hydrogels enabled sustained release of endogenous PDGF-BB for up to 20days and were resistant to protease degradation. PL hydrogel stimulated pro-angiogenic activity by promoting human MSC growth and invasion in a 3D environment, and enhancing endothelial cell sprouting alone and

in co-culture with MSCs. When delivered in vivo, the combination of PL and human MSCs improved local tissue perfusion after 8days compared to controls when assessed with laser Doppler perfusion imaging in a murine model of hind limb ischemia. These results support the use of a PL hydrogel as a scaffold for MSC delivery to promote vascular regeneration. STATEMENT OF SIGNIFICANCE: Innovative strategies for improved retention and viability of mesenchymal stem cells (MSCs) are needed for cellular therapies. Human platelet lysate is a potent serum supplement that improves the expansion of MSCs. Here we characterize our novel PL hydrogel's desirable structural and biologic properties for human MSCs and endothelial cells. PL hydrogel can localize cells for retention in the desired tissue, improves cell viability, and augments MSCs' angiogenic activity. As a result of these unique traits, PL hydrogel is ideally suited to serve as a cell delivery vehicle for MSCs injected into ischemic tissues to promote vascular regeneration, as demonstrated here in a murine model of hindlimb ischemia.

Saleh, F. A., et al. (2011). "Regulation of mesenchymal stem cell activity by endothelial cells." Stem Cells Dev **20**(3): 391-403.

Emerging data suggest that mesenchymal stem cells (MSCs) are part of a periendothelial niche, suggesting the existence of heterotypic cell-cell crosstalk between endothelial cells and MSCs that regulate MSCs in their local microenvironment. We determined the effects of paracrine factors secreted by human umbilical vein endothelial cells (HUVECs) on MSC survival, proliferation, and differentiation by using an optimized, serum-free HUVEC-conditioned medium (CM). HUVEC-CM induced a significant increase in the size and number of colony-forming units-fibroblast (CFU-F) and CFU-osteoblast (CFU-O) and stimulated the proliferation of MSCs as determined by 5-bromo-2'-deoxyuridine incorporation, compared with non-CM. We also demonstrated that CM significantly enhanced the osteogenic differentiation of MSCs as shown by alkaline phosphatase enzyme histochemistry and von Kossa staining of mineralized nodules as well as by quantitative reverse transcriptase-polymerase chain reaction analysis of osteogenic markers. In contrast, there was no effect on the adipogenic differentiation of MSCs. Bioinformatic integration of HUVEC and MSC gene expression datasets identified several candidate signaling pathways responsible for mediating these effects, including fibroblast growth factor, Wnt, bone morphogenetic protein, and Notch. These data suggest strongly that endothelial cells secrete a soluble factor (or factors) that stimulates progenitor cell activity and, selectively, the osteogenic differentiation of MSCs that could contribute to niche exit.

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

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

cocktails and/or 3D-based delivery strategies may be beneficial for bone repair.

Sales, V. L., et al. (2007). "Endothelial progenitor and mesenchymal stem cell-derived cells persist in tissue-engineered patch in vivo: application of green and red fluorescent protein-expressing retroviral vector." Tissue Eng **13**(3): 525-535.

An unresolved question regarding tissue-engineered (TE) cardiac valves and vessels is the fate of the transplanted cells in vivo. We have developed a strategy to track the anatomic location of seeded cells within TE constructs and neighboring tissues using a retroviral vector system encoding green and red fluorescent proteins (GFPs and RFPs, respectively) in ovine circulating endothelial progenitor cells (EPCs) and bone marrow-derived mesenchymal stem cells (BMSCs). We demonstrate that stable transduction ex vivo with high-titer Moloney murine leukemia virus-based retroviral vector yields transduction efficiency of greater than 97% GFP(+) EPC- and RFP(+) mesenchymal stem cell (MSC)-derived cells. Cellular phenotype and transgene expression were also maintained through 25 subsequent passages. Using a retroviral vector system to distinguish our pre-seeded cells from tissue-resident progenitor cells and circulating endothelial and marrow-derived precursors, we simultaneously co-seeded 2 x 10(6) GFP(+) EPCs and 2 x 10(5) RFP(+) MSCs onto the TE patches. In a series of ovine pulmonary artery patch augmentation studies, transplanted GFP(+) EPC- and RFP(+) MSC-derived cells persisted within the TE patch 7 to 14 days after implantation, as identified using immunofluorescence. Analysis showed 81% luminal coverage of the TE patches before implantation with transduced cells, increasing to 96% at day 7 and decreasing to 67% at day 14 post-implantation. This suggests a temporal association between retroviral expression of progenitor cells and mediating effects of these cells on the physiological remodeling and maturation of the TE constructs. To our knowledge, this is the first cardiovascular tissue-engineering in vivo study using a double-labeling method to demonstrate a direct evidence of the source, persistence, and incorporation into a TE vascular patch of co-cultured and simultaneously pre-seeded adult progenitor cells.

Salvolini, E., et al. (2010). "Skin-derived mesenchymal stem cells (S-MSCs) induce endothelial cell activation by paracrine mechanisms." Exp Dermatol **19**(9): 848-850.

The mesenchymal stem cells (MSCs) are able to accumulate at the site of tissue damage. For this reason, they must transmigrate across the endothelium. In this study, we focused on skin-derived MSCs (S-MSCs), because the skin represents a useful stem cell source, and we analysed the VEGF released by S-MSCs, because it is known to promote endothelial cell proliferation and vascular permeability. Moreover, we evaluated the influence of S-MSC-conditioned medium on human aortic endothelial cell intracellular calcium concentration ([Ca(2+)](i)) and nitric oxide (NO) production, given their important role in endothelial permeability modulation. Our results suggest that human S-MSCs may interact with the endothelium via paracrine mechanisms, probably leading to an alteration of the endothelial barrier. Consequently, we could hypothesize that a therapeutic approach based on human skin-derived MSCs may have a positive effect on tissue repair.

Sasaki, J., et al. (2015). "Fabrication of Biomimetic Bone Tissue Using Mesenchymal Stem Cell-Derived Three-Dimensional Constructs Incorporating Endothelial Cells." PLoS One **10**(6): e0129266.

The development of technologies to promote vascularization of engineered tissue would drive major developments in tissue engineering and regenerative medicine. Recently, we succeeded in fabricating three-dimensional (3D) cell constructs composed of mesenchymal stem cells (MSCs). However, the majority of cells within the constructs underwent necrosis due to a lack of nutrients and oxygen. We hypothesized that incorporation of vascular endothelial cells would improve the cell survival rate and aid in the fabrication of biomimetic bone tissues in vitro. The purpose of this study was to assess the impact of endothelial cells combined with the MSC constructs (MSC/HUVEC constructs) during short- and long-term culture. When human umbilical vein endothelial cells (HUVECs) were incorporated into the cell constructs, cell viability and growth factor production were increased after 7 days. Furthermore, HUVECs were observed to proliferate and self-organize into reticulate porous structures by interacting with the MSCs. After long-term culture, MSC/HUVEC

constructs formed abundant mineralized matrices compared with those composed of MSCs alone. Transmission electron microscopy and qualitative analysis revealed that the mineralized matrices comprised porous cancellous bone-like tissues. These results demonstrate that highly biomimetic bone tissue can be fabricated in vitro by 3D MSC constructs incorporated with HUVECs.

Schultheiss, J., et al. (2011). "Mesenchymal stem cell (MSC) and endothelial progenitor cell (EPC) growth and adhesion in six different bone graft substitutes." Eur J Trauma Emerg Surg **37**(6): 635-644.

INTRODUCTION: Several different synthetic and allograft bone graft substitutes are used clinically to treat large bone defects. In contrast to the "gold standard" of autologous bone grafts, these do not contain bone-forming (MSC) or vessel-forming (EPC) cells. In order to achieve the same level of success enjoyed by autologous bone grafts, they must be compatible with mesenchymal stem cells (MSC) and endothelial progenitor cells (EPC). In a previous study, we seeded MSC onto six different bone graft substitutes and then measured the cell adhesion, viability, differentiation, and morphology. In the present study, we seeded both MSC and EPC onto the same six bone graft substitutes and measured the same parameters. METHODS: In vitro, 125,000 MSC and 125,000 EPC were seeded onto Chronos((R)), Vitoss((R)), Actifuse((R)), Biobase((R)), Cerabone((R)), and Tutoplast((R)). Cell adhesion (fluorescence microscopy) and viability (MTT assay) were measured on days 2, 6, and 10. Osteogenic (cbfa-1, alkaline phosphatase [ALP], osteocalcin, collagen-1 alpha [Col1A]) and endothelial (von Willebrand factor [vWF], vascular endothelial growth factor [VEGF], kinase domain receptor [KDR]) gene expression were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). Morphology was described by scanning electron microscopy (SEM) at day 2. RESULTS: MSC adhered significantly better to Tutoplast((R)), Chronos((R)), Actifuse((R)), and Biobase((R)). EPC adhered better to Actifuse((R)), Chronos((R)), Biobase((R)), and Tutoplast((R)). Viability increased over time when seeded on Tutoplast((R)) and Chronos((R)). Osteogenic and endothelial gene expression were detectable at day 10 in cells seeded on Chronos((R)), Actifuse((R)), and Tutoplast((R)). The best morphology of MSC and EPC was found on Tutoplast((R)), Chronos((R)), Actifuse((R)), and Biobase((R)). CONCLUSION: When bone graft substitutes are used to help fill large defects, it is important that their interaction with these cells be supportive of bone healing.

Seebach, C., et al. (2012). "Endothelial progenitor cells improve directly and indirectly early vascularization of mesenchymal stem cell-driven bone regeneration in a critical bone defect in rats." Cell Transplant **21**(8): 1667-1677.

Early vascularization of a composite in a critical bone defect is a prerequisite for ingrowth of osteogenic reparative cells to regenerate bone, since lack of vessels does not ensure a sufficient nutritional support of the bone graft. The innovation of this study was to investigate the direct and indirect effects of endothelial progenitor cells (EPCs) and cotransplanted mesenchymal stem cells (MSCs) on the in vivo neovascularization activity in a critical size defect at the early phase of endochondral ossification. Cultivated human EPCs and MSCs were loaded onto beta-TCP in vitro. A critical-sized bone defect (5 mm) was created surgically in the femoral diaphysis of adult athymic rat and stabilized with an external fixateur. The bone defects were filled with beta-TCP, MSCs seeded on beta-TCP, EPCs seeded on beta-TCP, and coculture of MSCs and EPCs seeded on beta-TCP or autologous bone of rat. After 1 week, the rats were sacrificed. Using quantitative CD34 immunohistochemistry as well as qualitative analysis of vascularization (staining of MHC and VEGF) in decalcified serial sections were performed by means of an image analysis system. Fluorescence microscopy analyzed the direct effects and indirect effects of human implanted EPCs for vessel formation at bone regeneration site. Formation of a primitive vascular plexus was also detectable in the beta-TCP, MSC, or autologous bone group, but on a significantly higher level if EPCs alone or combined with MSCs were transplanted. Moreover, highest amount of vascularization were detected when EPCs and MSCs together were implanted. Early vascularization is improved by transplanted EPCs, which formed new vessels directly. Indeed the indirect effect of EPCs to vascularization is much higher. Transplanted EPC release chemotactic factors (VEGF) to recruit EPCs of the host and stimulate vascularization in the bone defect.

Transplantation of human EPCs displays a promising approach to improve early vascularization of a scaffold in a critical bone defect. Moreover, coculture of EPCs and MSCs demonstrate also a synergistic effect on new vessel formation and seems to be a potential osteogenic construct for in vivo application.

Shah, S. and K. T. Kang (2018). "Two-Cell Spheroid Angiogenesis Assay System Using Both Endothelial Colony Forming Cells and Mesenchymal Stem Cells." Biomol Ther (Seoul) **26**(5): 474-480.

Most angiogenesis assays are performed using endothelial cells. However, blood vessels are composed of two cell types: endothelial cells and pericytes. Thus, co-culture of two vascular cells should be employed to evaluate angiogenic properties. Here, we developed an in vitro 3-dimensional angiogenesis assay system using spheroids formed by two human vascular precursors: endothelial colony forming cells (ECFCs) and mesenchymal stem cells (MSCs). ECFCs, MSCs, or ECFCs+MSCs were cultured to form spheroids. Sprout formation from each spheroid was observed for 24 h by real-time cell recorder. Sprout number and length were higher in ECFC+MSC spheroids than ECFC-only spheroids. No sprouts were observed in MSC-only spheroids. Sprout formation by ECFC spheroids was increased by treatment with vascular endothelial growth factor (VEGF) or combination of VEGF and fibroblast growth factor-2 (FGF-2). Interestingly, there was no further increase in sprout formation by ECFC+MSC spheroids in response to VEGF or VEGF+FGF-2, suggesting that MSCs stimulate sprout formation by ECFCs. Immuno-fluorescent labeling technique revealed that MSCs surrounded ECFC-mediated sprout structures. We tested vatalanib, VEGF inhibitor, using ECFC and ECFC+MSC spheroids. Vatalanib significantly inhibited sprout formation in both spheroids. Of note, the IC50 of vatalanib in ECFC+MSC spheroids at 24 h was 4.0 +/- 0.40 microM, which are more correlated with the data of previous animal studies when compared with ECFC spheroids (0.2 +/- 0.03 microM). These results suggest that ECFC+MSC spheroids generate physiologically relevant sprout structures composed of two types of vascular cells, and will be an effective pre-clinical in vitro assay model to evaluate pro- or anti-angiogenic property.

Shin, Y. J., et al. (2015). "Expression of vascular endothelial growth factor-C (VEGF-C) and its receptor (VEGFR-3) in the glial reaction elicited by human mesenchymal stem cell engraftment in the normal rat brain." J Histochem Cytochem **63**(3): 170-180.

To determine whether vascular endothelial growth factor-C (VEGF-C) and its receptor (VEGFR-3) are involved in the glial reaction elicited by transplanted mesenchymal stem cells (MSCs), we examined the cellular localization of VEGF-C and VEGFR-3 proteins in the striatum of adult normal rats that received bone marrow-derived human MSCs. The MSC grafts were infiltrated with activated microglia/macrophages and astrocytes over a 2-week period post-transplantation, which appeared to parallel the loss of transplanted MSCs. VEGF-C/VEGFR-3 was expressed in activated microglia/macrophages recruited to the graft site, where the induction of VEGF-C protein was rather late compared with that of its receptor. VEGF-C protein was absent or very weak on day 3, whereas VEGFR-3 immunoreactivity was evident within the first three days. Furthermore, within three days, VEGF-C could be detected in the brain macrophages localized immediately adjacent to the needle track. At the same time, almost all the brain macrophages in both regions expressed VEGFR-3. Reactive astrocytes at the graft site expressed VEGFR-3, but not VEGF-C. These data demonstrated the characteristic time- and cell-dependent expression patterns for VEGF-C and VEGFR-3 within the engrafted brain tissue, suggesting that they may contribute to neuroinflammation in MSC transplantation, possibly through the recruitment and/or activation of microglia/macrophages and astrogliosis.

Smadja, D. M., et al. (2015). "Treprostinil indirectly regulates endothelial colony forming cell angiogenic properties by increasing VEGF-A produced by mesenchymal stem cells." Thromb Haemost **114**(4): 735-747.

Pulmonary vasodilators and prostacyclin therapy in particular, have markedly improved the outcome of patients with pulmonary hypertension (PH). Endothelial dysfunction is a key feature of PH, and we previously reported that treprostinil therapy increases number and proliferative potential of

endothelial colony forming cells (ECFC) isolated from PH patients' blood. In the present study, the objective was to determine how treprostinil contributes to the proangiogenic functions of ECFC. We examined the effect of treprostinil on ECFC obtained from cord blood in terms of colony numbers, proliferative and clonogenic properties in vitro, as well as in vivo vasculogenic properties. Surprisingly, treprostinil inhibited viability of cultured ECFC but did not modify their clonogenic properties or the endothelial differentiation potential from cord blood stem cells. Treprostinil treatment significantly increased the vessel-forming ability of ECFC combined with mesenchymal stem cells (MSC) in Matrigel implanted in nude mice. In vitro, ECFC proliferation was stimulated by conditioned media from treprostinil-pretreated MSC, and this effect was inhibited either by the use of VEGF-A blocking antibodies or siRNA VEGF-A in MSC. Silencing VEGF-A gene in MSC also blocked the pro-angiogenic effect of treprostinil in vivo. In conclusion, increased VEGF-A produced by MSC can account for the increased vessel formation observed during treprostinil treatment. The clinical relevance of these data was confirmed by the high level of VEGF-A detected in plasma from patients with paediatric PH who had been treated with treprostinil. Moreover, our results suggest that VEGF-A level in patients could be a surrogate biomarker of treprostinil efficacy.

Song, B. W., et al. (2015). "1H-pyrrole-2,5-dione-based small molecule-induced generation of mesenchymal stem cell-derived functional endothelial cells that facilitate rapid endothelialization after vascular injury." Stem Cell Res Ther **6**: 174.

INTRODUCTION: Despite the success of interventional processes such as drug-eluting stents, complete prevention of restenosis is still hindered by impaired or delayed endothelialization or both. Here, we report that 1H-pyrrole-2,5-dione-based small molecule-generated mesenchymal stem cell-derived functional endothelial cells (MDFECs) facilitated rapid transmural coverage of injured blood vessels. METHODS: Small molecules that induced CD31 expression were screened by principal component analysis (PCA). Rat mesenchymal stem cells (MSCs) were treated with selected small molecules for up to 16 days, and the expression levels of CD90 and CD31 were examined by immunocytochemistry. In vitro functional assays of MDFECs, including tube formation assays and nitric oxide production assays, were performed. After MDFECs (intravenous, 3x10(6) cells per animal) were injected into balloon-injured rats, neointima formation was monitored for up to 21 days. The endothelial coverage of denuded blood vessels was evaluated by Evans Blue staining. The functionality of repaired blood vessels was evaluated by measuring vasorelaxation and hemodynamic changes. Additionally, derivatives of the selected small molecules were examined for their ability to induce endothelial markers. RESULTS: PCA indicated that 3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione effectively induced MDFECs. MDFECs inhibited the neointima formation of denuded blood vessels by facilitating more rapid endothelialization. Further examination indicated that derivatives with a 1H-pyrrole-2,5-dione moiety are important for initiating the endothelial cell differentiation of MSCs. CONCLUSIONS: Small molecules with 1H-pyrrole-2,5-dione as a core structure have great potential to improve the efficacy of MSC-based cell therapy for vascular diseases, such as atherosclerosis and restenosis.

Song, Y. S., et al. (2017). "Bone marrow mesenchymal stem cell-derived vascular endothelial growth factor attenuates cardiac apoptosis via regulation of cardiac miRNA-23a and miRNA-92a in a rat model of myocardial infarction." PLoS One **12**(6): e0179972.

Bone marrow-mesenchymal stem cell (BM-MSC) therapy improves the recovery of cardiac function after myocardial infarction (MI); however, the underlying molecular mechanisms are not completely understood. Recent studies have shown that microRNAs (miRNAs) modulate the pathophysiology of cardiovascular diseases. Here, we investigated the mechanisms underlying the effects of BM-MSC-derived paracrine factors and cardiac miRNAs on myocardial regeneration after MI. In our study, MI was induced by permanent ligation of the left anterior descending (LAD) coronary artery. BM-MSCs transplanted in infarcted rats significantly downregulated the expression of miRNA-23a and miRNA-92a and inhibited apoptosis in the myocardium. An in vitro experiment showed that supernatant from BM-MSCs cultured under hypoxia contained higher levels of vascular

endothelial growth factor (VEGF) than that from BM-MSCs under normoxia. In addition, inhibition of miRNA-23a and miRNA-92a reduced cardiac apoptosis. Moreover, the VEGF-containing BM-MSC supernatant inhibited miRNA-23a and miRNA-92a expression and reduced apoptotic signaling in cardiomyocytes under hypoxia. These effects were inhibited when the supernatant was treated with neutralizing antibodies against VEGF. Our results indicate that the paracrine factor, VEGF, derived from transplanted BM-MSCs, regulated the expression of miRNAs such as miRNA-23a and miRNA-92a and exerted anti-apoptotic effects in cardiomyocytes after MI.

Tavakoli, F., et al. (2013). "Outcome improvement of cellular cardiomyoplasty using triple therapy: mesenchymal stem cell+erythropoietin+vascular endothelial growth factor." Eur J Pharmacol **714**(1-3): 456-463.

To improve cellular cardiomyoplasty efficacy after myocardial infarction (MI), we postulated that combining mesenchymal stem cells (MSCs) transplantation with anti-apoptotic and angiogenic effects of erythropoietin (EPO) and vascular endothelial growth factor (VEGF) may provide better prognosis in an infarcted heart 48 rats, underwent left anterior descending artery ligation, were divided into eight groups and treated as follows: Group 1: MSC+EPO+VEGF, Group 2: MSC+EPO, Group 3: MSC+VEGF, Group 4: MSC, Group 5: EPO+VEGF, Group 6: EPO, Group 7: VEGF and Group 8: Control. After MI induction, EPO and VEGF were injected subcutaneously at the dose of 3000 U/kg and 3 microg/kg respectively. MSCs were transplanted one week after MI. In the fourteenth and sixteenth days after infarction, EPO was injected again. Echocardiography demonstrated that all treatments improved left ventricular function significantly (before vs. after treatment) but in control group ejection fraction deteriorated over the 2-months period. Percent of ejection fraction recovery in all treatment groups were significantly greater than control ($P<0.05$). Compared with the control group, all treatments attenuated cell death in peri-infarct areas significantly, except groups 6 and 7. Vascular density of all treatment groups were more than control group but this superiority was statistically significant only in group 1 ($P<0.01$). All of our treatments had beneficial effects to some extent but MSC transplantation combined with EPO and VEGF administration resulted in superior therapeutic outcome in enhancing cell survival and neovascularization.

Trkov, S., et al. (2010). "Micropatterned three-dimensional hydrogel system to study human endothelial-mesenchymal stem cell interactions." J Tissue Eng Regen Med **4**(3): 205-215.

The creation of vascularized engineered tissues of clinically relevant size is a major challenge of tissue engineering. While it is known that endothelial and mural vascular cells are integral to the formation of stable blood vessels, the specific cell types and optimal conditions for engineered vascular networks are poorly understood. To this end, we investigated the vasculogenic potential of human mesenchymal stem cell (MSC) populations derived from three different sources: (a) bone marrow aspirates; (b) perivascular cells from the umbilical cord vein; and (c) perivascular cells from the umbilical cord artery. Cell populations were isolated and identified as MSCs according to their phenotypes and differentiation potential. Human umbilical vein endothelial cells (HUVECs) were used as a standard for endothelial cells. A novel co-culture system was developed to study cell-cell interactions in a spatially controlled three-dimensional (3D) fibrin hydrogel model. Using microfluidic patterning, it was possible to localize hydrogel-encapsulated HUVECs and MSCs within separate channels spaced at 500, 1000 or 2000 microm. All three MSC populations had similar expression profiles of mesenchymal cell markers and similar capacity for osteogenic and adipogenic differentiation. However, bone marrow-derived MSCs (but not umbilical vein or artery derived MSCs) showed strong distance-dependent migration toward HUVECs and supported the formation of stable vascular networks resembling capillary-like vasculature. The presented approach provides a simple and robust model to study the cell-cell communication of relevance to engineering vascularized tissues.

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

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

Wang, M., et al. (2009). "Signal transducer and activator of transcription 3-stimulated hypoxia inducible factor-1alpha mediates estrogen receptor-alpha-induced mesenchymal stem cell vascular endothelial growth factor production." J Thorac Cardiovasc Surg **138**(1): 163-171, 171 e161.

OBJECTIVE: Vascular endothelial growth factor, a critical factor in angiogenesis, mediates stem cell paracrine protective effects on ischemic myocardium. Studies on the role of sex in stem cell function have demonstrated that female mesenchymal stem cells produce greater vascular endothelial growth factor and provide better cardiac protection compared with male mesenchymal stem cells. The purpose of this study was to determine the mechanisms by which estrogen affects mesenchymal stem cell function as a potential therapeutic measure during ex vivo expansion, before therapeutic use. METHODS: A single-step purification method using adhesion to cell culture plastic was adopted to isolate mesenchymal stem cells from wild-type, estrogen receptor-alpha knockout, estrogen receptor-beta knockout, and signal transducer and activator of transcription 3 knockout mice. Mesenchymal stem cells were treated with or without 17beta-estradiol, estrogen receptor-alpha agonist (propyl pyrazoletriol), and estrogen receptor-beta agonist (diarylpropionitrile). RESULTS: 17beta-estradiol significantly increased mesenchymal stem cell vascular endothelial growth factor production in a dose-dependent manner. Both estrogen receptor-alpha and estrogen receptor-beta were expressed in mesenchymal stem cells. Administration of 17beta-estradiol or estrogen receptor-alpha agonist (not estrogen receptor-beta agonist) elevated mesenchymal stem cell vascular endothelial growth factor, hypoxia inducible factor-1alpha expression, and signal transducer and activator of transcription 3 activation. However, these effects were neutralized in estrogen receptor-alpha knockout mesenchymal stem cells, not estrogen receptor-beta knockout. Signal transducer and activator of transcription 3 knockout abolished estrogen receptor-alpha-induced hypoxia inducible factor-1alpha and subsequent vascular endothelial growth factor production. CONCLUSION: 17beta-estradiol-induced vascular endothelial growth factor production from mesenchymal stem cells appears to be mediated through estrogen receptor-alpha-activated signal transducer and activator of transcription 3-mediated hypoxia inducible factor-1alpha expression.

Wen, L., et al. (2016). "Role of Endothelial Progenitor Cells in Maintaining Stemness and Enhancing Differentiation of Mesenchymal Stem Cells by Indirect Cell-Cell Interaction." Stem Cells Dev **25**(2): 123-138.

A hot issue in current research regarding stem cells for regenerative medicine is the retainment of the stemness and multipotency of stem cell. Endothelial progenitor cells (EPCs) are characterized by an angiogenic switch that induces angiogenesis and further ameliorates the local microenvironment in ischemic organs. This study investigated whether EPCs could modulate the multipotent and differential abilities of mesenchymal stem cells (MSCs) in vitro and in vivo. We established an EPC/MSC indirect Transwell coculture system and then examined the

effects of EPCs on the regulation of MSC biological properties in vitro and bone formation in vivo. The in vitro studies showed that cocultured MSCs (coMSCs) display no overt changes in cell morphology but an enhanced MSC phenotype compared with monocultured MSCs (monoMSCs). Our studies regarding the cellular, molecular, and protein characteristics of coMSCs and monoMSCs demonstrated that EPCs greatly promote the proliferation and differentiation potentials of coMSCs under indirect coculture condition. The expression of the pluripotency factors OCT4, SOX2, Nanog, and Klf4 was also upregulated in coMSCs. Furthermore, coMSCs combined with fibrin glue showed improved bone regeneration when used to repair rat alveolar bone defects compared with monoMSC grafts in vivo. This study is the first to demonstrate that EPCs have dynamic roles in maintaining MSC stemness and regulating MSC differentiation potential.

Wen, L., et al. (2014). "[Effect of rat endothelial progenitor cell on proliferation and apoptosis of bone marrow mesenchymal stem cell]." Zhonghua Kou Qiang Yi Xue Za Zhi **49**(2): 106-110.

OBJECTIVE: To investigate the effect of endothelial progenitor cell (EPC) on regulating proliferation and apoptosis of bone marrow mesenchymal stem cell (BMSC) in the indirect co-culture system. METHODS: BMSC and EPC were cultivated and identified in vitro.Using transwell inserts to establish EPC and BMSC indirect co-culture system.Experimental groups were prepared as follows: BMSC/EPC indirect co-culture: BMSC were co-cultured with EPC that were separated in transwell insert. CONTROL: BMSC/BMSC were indirect co-cultured at the same cell counts with the experimental groups. Colony forming unit-fibroblast(CFU-F) assay was studied with three types of EPC/BMSC ratios (1: 1, 10: 1, 100: 1) to assess the capacity and efficiency for cell self-renewal.In addition, flow cytometry technique was used to detect the cell cycle at co-cultured 3 days and the cell apoptosis at co-cultured 3, 7, 10 d. RESULTS: Under indirect co-culture condition,EPC could significantly promote cell cycle progress and enhance capacity of cell self-renewal. Co-cultured EPC resulted in an accumulation of BMSC at S phase [experimental group: (15.72 +/- 2.93)%, control group: (2.02 +/- 0.66)%, P < 0.01]. CFU-F assay showed that the self-renewal capacity of EPC: BMSC/10: 1 group [(50.98 +/- 6.32)%] and 100:1 group[(57.87 +/- 14.06)%] were significantly higher than that in control group [(33.07 +/- 9.60)% and (30.06 +/- 7.20)%](P < 0.01). However,EPC had a slight but non-significant down-regulated effect on BMSC apoptosis(P > 0.05). CONCLUSIONS: Under indirect co-culture condition, EPC could enhance the proliferation of BMSC, but could not regulate cell apoptosis in vitro.

Wingate, K., et al. (2012). "Compressive elasticity of three-dimensional nanofiber matrix directs mesenchymal stem cell differentiation to vascular cells with endothelial or smooth muscle cell markers." Acta Biomater **8**(4): 1440-1449.

The importance of mesenchymal stem cells (MSC) in vascular regeneration is becoming increasingly recognized. However, few in vitro studies have been performed to identify the effects of environmental elasticity on the differentiation of MSC into vascular cell types. Electrospinning and photopolymerization techniques were used to fabricate a three-dimensional (3-D) polyethylene glycol dimethacrylate nanofiber hydrogel matrix with tunable elasticity for use as a cellular substrate. Compression testing demonstrated that the elastic modulus of the hydrated 3-D matrices ranged from 2 to 15 kPa, similar to the in vivo elasticity of the intima basement membrane and media layer. MSC seeded on rigid matrices (8-15 kPa) showed an increase in cell area compared with those seeded on soft matrices (2-5 kPa). Furthermore, the matrix elasticity guided the cells to express different vascular-specific phenotypes with high differentiation efficiency. Around 95% of MSC seeded on the 3-D matrices with an elasticity of 3 kPa showed Flk-1 endothelial markers within 24h, while only 20% of MSC seeded on the matrices with elasticity >8 kPa demonstrated Flk-1 marker. In contrast, approximately 80% of MSC seeded on 3-D matrices with elasticity >8 kPa demonstrated smooth muscle alpha-actin marker within 24h, while fewer than 10% of MSC seeded on 3-D matrices with elasticity <5 kPa showed alpha-actin markers. The ability to control MSC differentiation into either endothelial or smooth muscle-like cells based purely on the local elasticity of the substrate could be a powerful tool for vascular tissue regeneration.

Wu, B., et al. (2016). "Norepinephrine inhibits mesenchymal stem cell chemotaxis migration by increasing stromal cell-derived factor-1 secretion by vascular endothelial cells via NE/abrd3/JNK pathway." Exp Cell Res **349**(2): 214-220.

Mesenchymal stem cells (MSCs), which are physiologically maintained in vascular endothelial cell (VEC)-based niches, play a critical role in tissue regeneration. Our previous studies demonstrated that sympathetic denervation could promote MSC mobilization, thereby enhancing bone formation in distraction osteogenesis (DO), a self-tissue engineering for craniofacial and orthopeadic surgeries. However, the mechanisms on how sympathetic neurotransmitter norepinephrine (NE) regulates MSC migration are not well understood. Here we showed that deprivation of NE by transection of cervical sympathetic trunk (TCST) inhibited stromal cell-derived factor-1 (SDF-1) expression in the perivascular regions in rat mandibular DO. In vitro studies showed that NE treatment markedly upregulated p-JNK and therefore stimulated higher SDF-1 expression in VECs than control groups, and siRNA knockdown of the abrd3 gene abolished the NE-induced p-JNK activation. On the other hand, osteoblasts differentiated from MSCs showed an increase in SDF-1 secretion with lack of NE. Importantly, NE-treated VECs inhibited the MSC chemotaxis migration along the SDF-1 concentration gradient as demonstrated in a novel 3-chamber Transwell assay. Collectively, our study suggested that NE may increase the SDF-1 secretion by VECs via NE/abrd3/JNK pathway, thereby inhibiting the MSC chemotaxis migration from perivascular regions toward bone trabecular frontlines along the SDF-1 concentration gradient in bone regeneration.

Yang, J., et al. (2007). "[Differentiation of QY1 bone marrow pluripotential mesenchymal stem cell line cells into cardiomyocytes and vascular endothelial cells in vitro]." Zhong Nan Da Xue Xue Bao Yi Xue Ban **32**(1): 93-98.

OBJECTIVE: To explore the differentiation potential of QY1 bone marrow mesenchymal stem cell (MSCs) line cells into cardiacmyocytes and vascular endothelial cells in vitro, to optimize the suitable conditions of MSCs differentiating into cardiomyocytes in vitro, and to examine the potentials of MSCs differentiating into cardiomyogenesis and vasculogenesis. METHODS: Specifically committed differentiation inductive medium was employed, including 5-azacytidine for cardiomyogenesis and vascular endothelial growth factor for vasculogenesis in culture respectively in vitro. The differentiated cells were identified by immunohistochemistry and molecular biology. RESULTS: MSCs line cells had been cultured in the normal culture medium for 72 hours, then the differentiation inductive medium including 10 micromol/L 5-azacytidine was added into the normal culture dishes for 24 hours only. After that the culture medium was changed back to the normal culture medium. Normal culture medium was changed every 7 days. The second induction was performed after 14 days. The differentiated cells treated with 5-azacytidine could beat spontaneously and formed myotube structures in the optimal induction conditions, and the differentiation rate was (39.47+/-0.56)%. The differentiated cells expressed specific cardiomyocytic proteins identified by the positive immunohistochemistry staining with anti-alpha-sarcomeric antibody and anti-Cx-43 antibody, and also expressed the alpha-myosin heavy chain examined by RT-PCR. The differentiated cells began to appear as the lined up vascular endothelial cells after 48 hour treatment with vascular endothelial growth factor. Some of the differentiated cells connected each other to form vascular endothelial web-like structure after 7 day treatment with vascular endothelial growth factor. On 14 d after treating with vascular endothelial growth factor, the differentiated cells were identified by immunohistochemistry staining. The expressions of both specific surface antibody CD31 and factor VIII for vascular endothelial cells were positive. CONCLUSION: The cells of QY1 bone marrow mesenchymal stem cell line may differentiate into cardiomyocytes or vascular endothelial cells in vitro under specific condition.

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

Mesenchymal stem cell (MSC) transplantation has been proposed as a potential therapeutic approach for ischemic heart disease, but the regenerative capacity of these cells decreases

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

Yazdani, Y., et al. (2016). "Genistein Suppression of Matrix Metalloproteinase 2 (MMP-2) and Vascular Endothelial Growth Factor (VEGF) Expression in Mesenchymal Stem Cell Like Cells Isolated from High and Low Grade Gliomas." Asian Pac J Cancer Prev **17**(12): 5303-5307.

Objective: Brain tumors cause great mortality and morbidity worldwide, and success rates with surgical treatment remain very low. Several recent studies have focused on introduction of novel effective medical therapeutic approaches. Genistein is a member of the isoflavonoid family which has proved to exert anticancer effects. Here we assessed the effects of genistein on the expression of MMP-2 and VEGF in low and high grade gliomas in vitro. Materials and Methods: High and low grade glioma tumor tissue samples were obtained from a total of 16 patients, washed with PBS, cut into small pieces, digested with collagenase type I and cultured in DMEM containing 10% FBS. When cells reached passage 3, they were exposed to genistein and MMP-2 and VEGF gene transcripts were determined by quantitative real time PCR (qRT-PCR). Results: Expression of MMP-2 demonstrated 580-fold reduction in expression in low grade glioma cells post treatment with genistein compared to untreated cells (P value= 0.05). In cells derived from high grade lesions, expression of MMP-2 was 2-fold lower than in controls (P value> 0.05). Genistein caused a 4.7-fold reduction in VEGF transcript in high grade glioma cells (P value> 0.05) but no effects were evident in low grade glioma cells. Conclusion. Based on the data of the present study, low grade glioma cells appear much more sensitive to genistein and this isoflavone might offer an appropriate therapeutic intervention in these patients. Further investigation of this possibility is clearly warranted.

Yin, T., et al. (2016). "Malignant Pleural Effusion and ascites Induce Epithelial-Mesenchymal Transition and Cancer Stem-like Cell Properties via the Vascular Endothelial Growth Factor (VEGF)/Phosphatidylinositol 3-Kinase (PI3K)/Akt/Mechanistic Target of Rapamycin (mTOR) Pathway." J Biol Chem **291**(52): 26750-26761.

Malignant pleural effusion (PE) and ascites, common clinical manifestations in advanced cancer patients, are associated with a poor prognosis. However, the biological characteristics of malignant PE and ascites are not clarified. Here we report that malignant PE and ascites can induce a frequent epithelial-mesenchymal transition program and endow tumor cells with stem cell properties with high efficiency, which promotes tumor growth, chemoresistance, and immune evasion. We determine that this epithelial-mesenchymal transition process is mainly dependent on VEGF, one initiator of the PI3K/Akt/mechanistic target of rapamycin (mTOR) pathway. From the clinical observation, we define a therapeutic option with VEGF antibody for malignant PE and ascites. Taken together, our findings clarify a novel biological characteristic of malignant PE and ascites in cancer progression and

provide a promising and available strategy for cancer patients with recurrent/refractory malignant PE and ascites.

Yuan, Y., et al. (2016). "Mesenchymal stem cell-conditioned media ameliorate diabetic endothelial dysfunction by improving mitochondrial bioenergetics via the Sirt1/AMPK/PGC-1alpha pathway." Clin Sci (Lond) **130**(23): 2181-2198.

Vasculopathy is a major complication of diabetes. Impaired mitochondrial bioenergetics and biogenesis due to oxidative stress are a critical causal factor for diabetic endothelial dysfunction. Sirt1, an NAD(+)-dependent enzyme, is known to play an important protective role through deacetylation of many substrates involved in oxidative phosphorylation and reactive oxygen species generation. Mesenchymal stem cell-conditioned medium (MSC-CM) has emerged as a promising cell-free therapy due to the trophic actions of mesenchymal stem cell (MSC)-secreted molecules. In the present study, we investigated the therapeutic potential of MSC-CMs in diabetic endothelial dysfunction, focusing on the Sirt1 signalling pathway and the relevance to mitochondrial function. We found that high glucose-stimulated MSC-CM attenuated several glucotoxicity-induced processes, oxidative stress and apoptosis of endothelial cells of the human umbilical vein. MSC-CM perfusion in diabetic rats ameliorated compromised aortic vasodilatation and alleviated oxidative stress in aortas. We further demonstrated that these effects were dependent on improved mitochondrial function and up-regulation of Sirt1 expression. MSC-CMs activated the phosphorylation of phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt), leading to direct interaction between Akt and Sirt1, and subsequently enhanced Sirt1 expression. In addition, both MSC-CM and Sirt1 activation could increase the expression of peroxisome proliferator-activated receptor gamma co-activator-1alpha (PGC-1alpha), as well as increase the mRNA expression of its downstream, mitochondrial, biogenesis-related genes. This indirect regulation was mediated by activation of AMP-activated protein kinase (AMPK). Overall our findings indicated that MSC-CM had protective effects on endothelial cells, with respect to glucotoxicity, by ameliorating mitochondrial dysfunction via the PI3K/Akt/Sirt1 pathway, and Sirt1 potentiated mitochondrial biogenesis, through the Sirt1/AMPK/PGC-1alpha pathway.

Zanotti, L., et al. (2016). "Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1." Leukemia **30**(5): 1143-1154.

Mesenchymal stem cells (MSC) represent a promising therapeutic approach in many diseases in view of their potent immunomodulatory properties, which are only partially understood. Here, we show that the endothelium is a specific and key target of MSC during immunity and inflammation. In mice, MSC inhibit activation and proliferation of endothelial cells in remote inflamed lymph nodes (LNs), affect elongation and arborization of high endothelial venules (HEVs) and inhibit T-cell homing. The proteomic analysis of the MSC secretome identified the tissue inhibitor of metalloproteinase-1 (TIMP-1) as a potential effector molecule responsible for the anti-angiogenic properties of MSC. Both in vitro and in vivo, TIMP-1 activity is responsible for the anti-angiogenic effects of MSC, and increasing TIMP-1 concentrations delivered by an Adeno Associated Virus (AAV) vector recapitulates the effects of MSC transplantation on draining LNs. Thus, this study discovers a new and highly efficient general mechanism through which MSC tune down immunity and inflammation, identifies TIMP-1 as a novel biomarker of MSC-based therapy and opens the gate to new therapeutic approaches of inflammatory diseases.

Zhang, G., et al. (2008). "Arterial-venous endothelial cell fate is related to vascular endothelial growth factor and Notch status during human bone mesenchymal stem cell differentiation." FEBS Lett **582**(19): 2957-2964.

Human bone mesenchymal stem cells (hMSCs) can differentiate into endothelial cells (ECs), so we aimed to investigate whether hMSCs could also differentiate into a specific arterial or venous ECs. hMSCs were induced to differentiate into ECs using vascular endothelial growth factor (VEGF). Low VEGF concentration (50 ng/ml) upregulated the venous marker gene EphB4, however high concentration (100 ng/ml) upregulated the arterial marker genes ephrinB2, Dll4 and Notch4, and downregulated the venous marker genes EphB4 and COUP-TFll. This VEGF dose-dependent induction was largely blocked

by inhibition of the Notch pathway in hMSCs treated with gamma-secretase inhibitor. Therefore, differentiation of hMSCs into arterial- or venous-specific ECs depends on VEGF and is regulated by the Notch pathway.

Zhang, H., et al. (2014). "Endothelial progenitor cells as a possible component of stem cell niche to promote self-renewal of mesenchymal stem cells." Mol Cell Biochem **397**(1-2): 235-243.

Stem cells dwell at the "stem cell niche" to accomplish a series of biological processes. The composition of the niche should be determined because the insufficient understanding of this feature limits the development in the study of stem cells. We showed in our study on mesenchymal stem cells (MSCs) that the MSCs first neighbored to CD31(+) cells, which proved to be endothelial progenitor cells (EPCs), and formed a group of cell colony before they exerted their biological functions. It was further proved that EPCs have close interactions with MSCs and promoted the self-renewal of the MSCs in vitro and in vivo. Together with these achievements, we hypothesized that EPCs may be a possible biological component of the MSC stem cell niche and affect the biological processes of MSCs.

Zhang, H., et al. (2016). "Construction and characterization of osteogenic and vascular endothelial cell sheets from rat adipose-derived mesenchymal stem cells." Tissue Cell **48**(5): 488-495.

In this study, adipose-derived mesenchymal stem cells (ADSCs) were isolated from adipose tissues of rats. Flow cytometry identification showed that ADSCs of passage 3 highly expressed CD29 and CD44, but hardly expressed CD31 and CD45. Adipogenic, osteogenic, and chondrogenic differentiation were confirmed by the results of oil red O staining, alkaline phosphatase (ALP), and alcian blue staining, respectively. ADSCs at a density of 1x10(6)/cm(2) were cultured in the osteogenic medium and the osteogenic cell sheets could be obtained after 14 d. The cell sheets were positive with von kossa staining. The transmission electron microscopy (TEM) result showed that needle-like calcium salt crystals were deposited on the ECM. These results suggested that the osteogenic cell sheets may have potential osteogenesis ability. ADSCs at a density of 1x10(6)/cm(2) were cultured in the endothelial cell growth medium-2 and the endothelial cell sheets can be formed after 16 d of culture. The TEM image confirmed that the Weibel-Palade corpuscle was seen in the cells. The expression of CD31 was positive, suggesting that the endothelial cell sheets may have a strong ability to form blood vessels. In this study, two types of cell sheets with the potential abilities of osteogenesis and blood vessels formation were obtained by induced culture of ADSCs in vitro, which lays a foundation to build vascularized tissue engineered bone for the therapy of bone defects.

Zhang, J., et al. (2011). "[Effect of vascular endothelial growth factor on bone marrow-derived mesenchymal stem cell proliferation and the signaling mechanism]." Nan Fang Yi Ke Da Xue Xue Bao **31**(10): 1697-1700.

OBJECTIVE: To observe the effect of vascular endothelial growth factor (VEGF) on bone marrow-derived mesenchymal stem cell (MSC) proliferation and explore the signaling mechanism involved. METHODS: MSC culture was performed following the classical whole bone marrow adhering method. The characteristics of MSC were identified by induction of multi-lineage differentiation and flow cytometry for surface marker analysis (CD34, CD45, CD29, and CD90). Following the addition of 50 nmol/L wortmannin, 50 micromol/L PD98059, 30 micromol/L SB203580, 10 micromol/L H89, 20 micromol/L Y27632, 1 micromol/L rapamycin, 10 micromol/L staurosporine, 6 nmol/L Go6976, or 50 micromol/L Pseudo Z inhibitors in the cell culture, the MSC were treated with 20 ng/ml VEGF and the changes of the cell proliferation rate was measured with MTT assay. RESULTS: Cultured MSC were capable of multi-linage differentiation and did not express VEGF-R, CD29 or CD90. Treatment with 20 ng/ml VEGF obviously promoted MSC proliferation, and this effect was inhibited partially by p38 mitogen-activated protein kinase (MAPK) inhibitor rapamycin, PD98059, SB203580, Go6976, and staurosporine. CONCLUSIONS: VEGF promotes MSC proliferation in close relation to the AKT-PKC pathway, in which PKC signal pathway may play the central role.

Zhang, Z., et al. (2014). "Endothelial cell-secreted EGF induces epithelial to mesenchymal transition and endows head and neck cancer cells with stem-like phenotype." Cancer Res **74**(10): 2869-2881.

Emerging evidence suggests that endothelial cell-secreted factors contribute to the pathobiology of squamous cell carcinoma (SCC) by enhancing invasive migration and resistance to anoikis. Here, we report that SCC cells within the perivascular niche have undergone epithelial to mesenchymal transition (EMT) in a primary human SCC of a patient that developed distant metastases. Endothelial cell-secreted EGF induced EMT of human SCC cells in vitro and also induced acquisition of a stem-like phenotype. In vivo, tumor xenografts vascularized with EGF-silenced endothelial cells exhibited a smaller fraction of cancer stem-like cells (ALDH(+)CD44(+)) and were less invasive than tumors vascularized with control endothelial cells. Collectively, these results demonstrated that endothelial cell-EGF induces EMT and acquisition of stem-like properties by head and neck tumor cells. On this basis, we suggest that vascular endothelial cells contribute to tumor dissemination by secreting factors that endow carcinoma cells with enhanced motility and stemness.

Zhao, Y. F., et al. (2014). "Mesenchymal stem cell-based developmental endothelial locus-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice." Mol Med Rep **9**(5): 1583-1589.

Studies have suggested that bone marrow-derived mesenchymal stem cells (MSCs) may be used as a tool for gene therapy. Developmental endothelial locus-1 (Del-1) is a critical factor for cell migration and infiltration via the inhibition of the function of a major leukocyte adhesion receptor LFA-1 which prevents leukocyte adhesion to the endothelium. In the present study, we hypothesized that MSC-based Del-1 gene therapy may have potential therapeutic applications for lipopolysaccharide (LPS)-induced lung injury. The MSCs in the present assay were isolated from 6 week-old male mice. In order to investigate the therapeutic effect of the Del-1 gene on LPS-induced ALI mice, a lentivirus vector containing the Del-1 gene was constructed and transduced into the MSCs. In the in vivo assay, we induced lung injury with LPS injection and treated mice with different groups of MSCs, and compared with groups treated with MSCs alone, we observed that the administration with MSCs carrying Del-1 (MSCs-Del1) markedly alleviated the LPS-induced lung injury. There were significant decreases in the number of neutrophils in bronchoalveolar lavage (BAL) and the serum levels of TNF-alpha and IL-6 in the Del-1-expressed MSC-treated mice. Furthermore, compared with MSCs treated alone, Del1-MSC-treated mice also exhibited low lung injury scores, high protein concentrations and myeloperoxidase activity. In conclusion, treatment with Del-1-expressed MSCs significantly decreases the severity of endotoxin-induced acute lung injury and the level of inflammatory cytokines in mice.

Zhao, Z. L., et al. (2009). "[Migratory and chemoattractant responses of mesenchymal stem cells to oxidative stress injury of endothelial cell in vitro]." Zhonghua Yi Xue Za Zhi **89**(22): 1577-1581.

OBJECTIVE: To investigate the possibilities of human mesenchymal stem cells (hMSCs) migrating toward the oxidative stress injuries of endothelial cells. METHODS: hMSCs were isolated and cultured from human marrow in vitro and the multipotential differentiation of P3 hMSCs identified by specific medium induced to differentiate into osteoblasts, adipocytes and endothelial cells. And the marker antigen of P3 hMSCs was detected by flow cytometry (FCM) and immunohistochemistry. Then a cellular model of hMSCs migrating toward the oxidative stress injuries of endothelial cells was created, i. e. 1 x 10(5) hMSCs were seeded in Transwell upper chamber, indirectly co-cultured with ECV-304 cells seeded in the Transwell inferior chamber and was injured by adding 3% H2O2 into the medium (final concentration of 0.01 ml/ml) for 1 h, the injured ECV-304 cells + hMSCs group (n = 8), as experimental group, and in the mean time, hMSCs indirectly co-cultured with uninjured ECV-304 cells in Transwell chamber, ECV-304 cells + hMSCs group (n = 8) and hMSCs monoculture group (n = 8) in Transwell chamber as control groups. After a 12-h culture in all groups, the migrating hMSCs in Transwell upper chamber were HE-stained and counted under an inverted phase contrast microscope. To understand the reason why hMSCs migrated to the oxidative stress injured endothelial cells, ELISA was employed to measure the concentration of monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) of cellular supernatant in ECV-304 cells with H2O2 1-h treating group (H2O2 treatment group) or without H2O2 treating group (control group). RESULTS:

The multipotential differentiation experiment demonstrated that the cultured P3 hMSCs can be induced to differentiate in vitro into osteoblasts, adipocytes and endothelial cells. And the expressions of CD29, CD44, CD90 and CD106 were positive in hMSCs while CD31, CD34, CD45 and CD49b negative by using FCM and immunohistochemistry. And the effects of hMSCs upon in vitro movement toward oxidative stress injuries of ECV-304 cells were averaged (8. 00 +/- 0.22) cells/HP in the injured ECV-304 cells + hMSCs group, significantly higher than those of the ECV-304 cells + hMSCs group [(0.20 +/- 0.05) cells/HP, P < 0.01] and the hMSCs monoculture group [(0.00 +/- 0.00) cells/HP, P < 0.01). The concentrations of MCP-1 and VCAM-1 in cellular supernatant of the H2O2 treatment group were significantly higher than those of the control group [(69.2 +/- 3.5) ng/ml vs (62.5 +/- 3.6) ng/ml, P < 0.05; (114.0 +/- 7.5) ng/ml vs (97.2 +/- 5.0) ng/ml, P < 0.01]. CONCLUSIONS: The oxidative stress injuries of endothelial cells chemoattracted the hMSCs toward the injured site and its mechanism may be correlated with releasing a certain concentration of chemoattractant factor to result in the elevations of MCP-1 and VCAM-1 by oxidative stress injury.

Zhen, G., et al. (2010). "Mesenchymal stem cell transplantation increases expression of vascular endothelial growth factor in papain-induced emphysematous lungs and inhibits apoptosis of lung cells." Cytotherapy **12**(5): 605-614.

BACKGROUND: Pulmonary emphysema is characterized by loss of alveolar structures. We have found that bone marrow (BM) mesenchymal stem cell (MSC) transplantation ameliorates papain-induced pulmonary emphysema. However, the underlying mechanism is not completely understood. It has been shown that blocking the vascular endothelial growth factor (VEGF) signaling pathway leads to apoptosis of lung cells and pulmonary emphysema, and MSC are capable of secreting VEGF. We hypothesized that MSC transplantation may have a protective effect on pulmonary emphysema by increasing VEGF-A expression and inhibiting apoptosis of lung cells. METHODS: We examined the morphology and expression of VEGF-A in rat lung after papain treatment and MSC transplantation. We also used a co-culture system in which MSC and cells prepared from papain-treated lungs or control lungs were cultured together. The levels of VEGF-A in cells and culture medium were determined, and apoptosis of cultured lung cells was evaluated. RESULTS: VEGF-A expression in rat lungs was decreased after papain treatment, which was partly rescued by MSC transplantation. MSC production of VEGF-A was increased when MSC were co-cultured with cells prepared from papain-treated lungs. Furthermore, the apoptosis of papain-treated lung cells was inhibited when co-cultured with MSC. The induction of MSC production of VEGF-A by papain-treated lung cells was inhibited by adding anti-tumor necrosis factor (TNF)-alpha antibody to the medium. CONCLUSIONS: The protective effect of MSC transplantation on pulmonary emphysema may be partly mediated by increasing VEGF-A expression and inhibiting the apoptosis of lung cells. TNF-alpha released from papain-treated lung cells induces MSC to secret VEGF-A.

Zhou, L., et al. (2018). "Efficient differentiation of vascular endothelial cells from dermal-derived mesenchymal stem cells induced by endothelial cell lines conditioned medium." Acta Histochem.

OBJECTIVE: To directionally-differentiate dermis-derived mesenchymal stem cells (DMSCs) into vascular endothelial cells (VECs) in vitro, providing an experimental basis for studies on the pathogenesis and treatment of vascular diseases. METHODS: After separation by adherent culture, VEC line supernatant, vascular endothelial growth factor (VEGF), bone morphogenetic protein-4 and hypoxia were used for the differentiation of VECs from DMSCs. The cell type was authenticated by flow cytometry, matrigel angiogenesis assay in vitro, and immunofluorescent staining during differentiation. The VEGF concentration was investigated by enzyme-linked immunosorbent assay. RESULTS: After 28 days of differentiation, the cell surface marker CD31 was significantly positive (80%-90%) by flow cytometry in the VEC line-conditioned culture, which was significantly higher than in the other groups. Differentiated DMSCs had the ability to ingest Dil-ac-LDL and vascularize in the conditioned culture, but not in the other groups. In the VEC line supernatant, the concentration of VEGF was very low. The VEGF concentration changed along with the differentiation into VECs in the medium of the conditioned culture group.

CONCLUSION: VEC line supernatant can induce the differentiation of DMSCs into VECs, possibly through the pathway except VEGF.

Zhou, Z. and Z. You (2016). "Mesenchymal Stem Cells Alleviate LPS-Induced Acute Lung Injury in Mice by MiR-142a-5p-Controlled Pulmonary Endothelial Cell Autophagy." Cell Physiol Biochem **38**(1): 258-266.

BACKGROUND/AIMS: Damages of pulmonary endothelial cells (PECs) represent a critical pathological process during acute lung injury (ALI), and precede pulmonary epithelial cell injury, and long-term lung dysfunction. Transplantation of mesenchymal stem cells (MSCs) has proven therapeutic effects on ALI, whereas the underlying mechanisms remain ill-defined. METHOD: We transplanted MSCs in mice and then induced ALI using Lipopolysaccharides (LPS). We analyzed the changes in permeability index and lung histology. Mouse PECs were isolated by flow cytometry based on CD31 expression and then analyzed for autophagy-associated factors LC3 and Beclin-1 by Western blot. Beclin-1 mRNA was determined by RT-qPCR. In vitro, we performed bioinformatics analyses to identify the MSCs-regulated miRNAs that target Beclin-1, and confirmed that the binding was functional by 3'-UTR luciferase reporter assay. RESULTS: We found that MSCs transplantation significantly reduced the severity of LPS-induced ALI in mice. MSCs increased autophagy of PECs to promote PEC survival. MSCs increased Beclin-1 protein but not mRNA. MiR-142a-5p was found to target the 3'-UTR of Beclin-1 mRNA to inhibit its protein translation in PECs. MSCs reduced the levels of miR-142a-5p in PECs from LPS-treated mice. CONCLUSION: MSCs may alleviate LPS-ALI through downregulation of miR-142a-5p, which allows PECs to increase Beclin-1-mediated cell autophagy.

Zhu, F., et al. (2010). "Effects of bone marrow-derived mesenchymal stem cells engraftment on vascular endothelial cell growth factor in lung tissue and plasma at early stage of smoke inhalation injury." World J Emerg Med **1**(3): 224-228.

BACKGROUND: This study was undertaken to determine the effect of mesenchymal stem cells (MSCs) engraftment on vascular endothelial cell growth factor (VEGF) in lung tissue, plasma and extravascular lung water at early stage of smoke inhalation injury. METHODS: A rabbit smoke inhalation injury model was established using a home-made smoke inhalation injury generator, and rabbits were divided into two groups randomly: a control group (S group, n=32) and a MSCs treatment group (M group, n=32). 10 ml PBS was injected via the ear marginal vein immediately at injury into the S group. Third generation MSCs with a concentration of 1x10(7)/10 ml PBS were injected via the ear marginal vein immediately at injury into the M group. VEGF in peripheral blood and lung tissue were measured at 0 (baseline), 2, 4 and 6 hours after injection respectively and analyzed. The right lungs of rabbits were taken to measure lung water mass fraction. RESULTS: In the lung tissue, VEGF decreased gradually in the S group ($P<0.05$) and significantly decreased in the M group ($P<0.05$), but it increased more significantly than the values at the corresponding time points ($P<0.05$). In peripheral blood, VEGF increased gradually in the S group ($P<0.05$) and markedly increased in the M group ($P<0.05$), but it decreased more significantly than the values at corresponding time points ($P<0.05$). CONCLUSION: MSCs engraftment to smoke inhalation injury could increase VEGF in lung tissue, decrease VEGF in plasma and reduce extravascular lung water, indicating its protective effect on smoke inhalation injury.

Zisa, D., et al. (2009). "Vascular endothelial growth factor (VEGF) as a key therapeutic trophic factor in bone marrow mesenchymal stem cell-mediated cardiac repair." Biochem Biophys Res Commun **390**(3): 834-838.

We recently demonstrated a novel effective therapeutic regimen for treating hamster heart failure based on injection of bone marrow mesenchymal stem cells (MSCs) or MSC-conditioned medium into the skeletal muscle. The work highlights an important cardiac repair mechanism mediated by the myriad of trophic factors derived from the injected MSCs and local musculature that can be explored for non-invasive stem cell therapy. While this therapeutic regimen provides the ultimate proof that MSC-based cardiac repair is mediated by the trophic actions independent of MSC differentiation or stemness, the trophic factors responsible for cardiac regeneration after MSC therapy remain largely undefined. Toward this aim, we took advantage of the finding that human and

porcine MSCs exhibit species-related differences in expression of trophic factors. We demonstrate that human MSCs when compared to porcine MSCs express and secrete 5-fold less vascular endothelial growth factor (VEGF) in conditioned medium (40+/-5 and 225+/-17 pg/ml VEGF, respectively). This deficit in VEGF output was associated with compromised cardiac therapeutic efficacy of human MSC-conditioned medium. Over-expression of VEGF in human MSCs however completely restored the therapeutic potency of the conditioned medium. This finding indicates VEGF as a key therapeutic trophic factor in MSC-mediated myocardial regeneration, and demonstrates the feasibility of human MSC therapy using trophic factor-based cell-free strategies, which can eliminate the concern of potential stem cell transformation.

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5/20/2024