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Mechanobiology of mesenchymal stem cells and their use in cardiovascular repair

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1. ABSTRACT

Mesenchymal stem cells (MSCs) derived from bone marrow have shown great promise in tissue repair. While these cells induce little immune response, they show marked self-renewal properties and can differentiate into many cell types. Recent evidence shows that mechanical factors such as fluid shear stress, mechanical strain and the rigidity of extracellular matrix can regulate the proliferation and differentiation of MSCs through various signaling pathways. Transplanted MSCs enhance angiogenesis and contribute to remodeling of the vasculature. In this review, we will focus on the responses of vascular cells and MSCs to shear stress, strain and matrix rigidity and will discuss the use of MSCs in myocardial repair and vascular tissue engineering.

2. INTRODUCTION

Bone marrow is one of the most abundant sources for adult stem cells and progenitor cells. Hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs) can be isolated from bone marrow (1-3). HSCs give rise to blood cells and are generally characterized by positively expressing the cell surface antigen CD34. These cells, along with EPCs, are mobilized in response to certain growth factors and cytokines released during vascular injury, and therefore, can be isolated from peripheral blood in addition to bone marrow. EPCs that express both CD34 and CD133 can differentiate into endothelial cells (ECs) and have been shown to enhance angiogenesis in injury and ischemic conditions (4, 5) as well as endothelialize vascular grafts

(6-8). Bone marrow MSCs, the pluripotent stromal cells derived from bone marrow, can be expanded a billion-fold in culture, and can be stimulated to differentiate into a variety of cell types (9-15). For example, MSCs transplanted into the heart can differentiate into smooth muscle cells (SMCs) and contribute to remodeling of the vasculature (16, 17). In addition, the transplantation of *ex vivo*-expanded allogeneic MSCs has shown little immunogenic responses *in vivo* (18). Unlike other cell types, MSCs do not express the major histocompatibility complex II (MHC II) antigens that are responsible for immune rejection, making MSCs a candidate cell source for allogeneic cell transplantation. Recent reports indicate that MSCs do not acquire MHC II cell surface antigens upon differentiation along adipogenic, chondrogenic and osteogenic lineages, and it is possible that MHC II antigens are not expressed upon cardiovascular differentiation as well (19). In addition, MSCs can modulate immune responses by suppressing both B and T cell functions (20, 21). Thus, MSCs are a promising cell source for cardiovascular tissue engineering.

MSCs are most commonly isolated from the bone marrow of the iliac crest, but they can also be found in umbilical cord blood, peripheral blood and various tissues including adipose tissue and blood vessels. To purify MSCs from bone marrow, the cells are plated on a culture dish and the adherent cells are cultured while the floating cells in the media, largely made up of hematopoietic cells, are discarded. For further purification, MSCs can be isolated through density centrifugation of bone marrow using a Percoll gradient before plating on a culture dish (22). However, adhesion to a culture dish is what truly separates MSCs from the other cell types in bone marrow. Although no single cell surface marker for MSCs exists, they are in general positive for STRO-1 (a stromal cell surface antigen), CD105 (endoglin, receptor for transforming growth factor-β (TGF-β) and integrins), CD29 (integrin β1), CD44 (receptor for hyaluronic acid and matrix proteins) and CD166 (a cell adhesion molecule), and negative for CD14 (monocyte surface antigen), CD34 (HSC surface antigen), and CD45 (leukocyte surface antigen) (23, 24). These and other cell surface markers, although not unique for MSCs, are commonly used to isolate and characterize MSCs by fluorescence-activated cell sorting (FACS). However, the specific roles of MSC markers in MSC function have not been well elucidated.

The pluripotency of MSCs has been demonstrated by their osteogenic, chondrogenic, myogenic and adipogenic potential in response to different cocktails of growth factors (9-15). Although growth factors have been shown to induce a certain lineage, for example 5-azacytidine for muscle (10), and dexamethasone, beta-glycerophosphate and ascorbic acid for bone (25), mechanical factors also play an important role in MSC differentiation. It is widely accepted that mechanical forces are important in development, growth, and the maintenance and function of tissues such as the production of bone, the contraction of muscle, the vibrations of the eardrum and the remodeling of cardiovascular tissues. The cells of the cardiovascular system experience a myriad of mechanical signals, from the fluid shear stress caused by the blood flow to the cyclic mechanical stretch in the myocardium and vessel walls. These signals regulate the functions of ECs, SMCs, myoblasts and cardiomyocytes. Recent research has been extended to the mechanobiology of stem cells and progenitor cells. These studies are not only important for the possibility of differentiating stem cells and progenitor cells into cardiovascular cells, but also critical for the understanding of the role of mechanical factors in cardiovascular development and remodeling. Furthermore, stem cells and progenitor cells can be used for cardiac therapies and vascular graft construction, both of which involve cell transplantation into cardiac and vascular tissues. Evidence has also shown that MSCs not only home to sites of vascular injury, but also reside in the medial layer of a normal healthy vessel as a subpopulation with SMCs (26). Therefore, it is essential to investigate the responses of stem cells and progenitor cells to cardiovascular mechanical factors. In this paper, we will review the studies on the mechanical regulation of cardiovascular cells and stem cells, particularly MSCs. We will also discuss the potential of MSCs for cardiovascular regeneration and tissue engineering.

3. MECHANOBIOLOGY OF MESENCHYMAL STEM CELLS

3.1. Effects of fluid shear stress on cardiovascular cells and mesenchymal stem cells

In terms of the mechanical environment of the vasculature, the inner surface of a blood vessel is constantly subjected to a fluid shear stress caused by blood flowing tangentially across the lumen surface. Under physiological conditions, only the EC monolayer is subjected to arterial levels of fluid shear stress (average ~10-20 dynes/cm^2) (27, 28). Flow channels have been used to investigate the responses of cultured ECs to shear stress *in vitro* because the chemical and mechanical factors can be well controlled. Such *in vitro* studies have shown that shear stress induces the remodeling of the EC monolayer, including the alignment of cells, the increase of stress fibers, the release of vasoactive substances, the decrease in cell proliferation, and the regulation of the expression of a variety of genes, such as c-fos, platelet-derived growth factor (PDGF), TGF-beta1, matrix metalloproteinases (MMPs) and intracellular adhesion molecule-1 (ICAM-1) (29-41). Shear stress also activates multiple signaling pathways, e.g., focal adhesion kinase (FAK), mitogen-activated protein kinase (MAPK), phosphoinositide-3 kinase (PI3-K), Akt and Rho family GTPases, and increases integrin clustering and integrin-ligand binding (42-55). Both *in vivo* and *in vitro* studies have shown that laminar shear stress can promote lamellipodial protrusion and EC migration in the flow direction (mechanotaxis) in wound healing. Furthermore, studies have also shown that different flow profiles, such as disturbed flow patterns, can lead to differential effects on EC migration (29, 56-62).

In contrast to vascular ECs, the effects of fluid shear stress on vascular SMCs are less clearly understood. SMCs populate the medial layer of a blood vessel and play important roles in the control of vasoactivity and the

remodeling of the vessel wall. Under physiological conditions, SMCs are embedded in a three-dimensional (3-D) extracellular matrix (ECM) and experience very low shear stress due to interstitial fluid flow (~1 dynes/cm^2 by theoretical estimation) (63). Under pathological conditions, ECs and the underlying elastic lamina are disrupted. As a result, SMCs migrate into the lumen and are directly exposed to fluid shear stress. At the same time, SMCs de-differentiate from a contractile to a proliferative phenotype, and SMC proliferation, migration and ECM synthesis contribute significantly to the inward remodeling of the vessel wall and the narrowing of the lumen (64-66). Shear stress has been shown to regulate EC-covered SMC proliferation in vascular grafts (67-70), but studies on the effects of fluid shear stress on SMC proliferation have generated mixed results (71-74). We have found that fluid shear stress increases human SMC proliferation by activating the PI 3-kinase/Akt pathway and that p21 is a downstream target of this pathway (Hsu, unpublished data). Our DNA microarray results indicate that fluid shear stress downregulates p21, p57 and Kruppel-like 4 (KLF4), upregulates cyclin D1, and significantly modulates the expression of numerous genes in human SMCs, such as growth factors, transcription factors, ECM, cell adhesion molecules, and intracellular signaling molecules.

In addition to vascular cells, a few studies have focused on the effects of hemodynamic stresses on cardiac cells. Fluid shear stress has been shown to regulate cardiac EC gene expression and cytoskeletal rearrangement (29). Hemodynamic stresses are required in the normal development of an embryonic zebrafish heart (75). *In vivo* imaging reveals a high shear, vertical flow profile at two key stages during the development of the heart. Furthermore, subsequent blockage of flow using beads results in abnormal heart development. A study on the role of fluid flow in regulating essential behaviors of neonatal cardiac myocytes demonstrated that shear stress applied by a fluid jet pulse could activate and propagate action potentials in these cells (76). Future studies focused on further characterizing the effects of fluid forces on cardiac cells could potentially lead to the development of improved therapeutic strategies for cardiovascular engineering.

Recently, the effects of shear stress on various types of stem cells and progenitor cells such as EPCs, embryonic stem cells (ESCs) and MSCs have been investigated. Shear stress has been shown to increase the proliferation, differentiation and capillary tube formation of EPCs, as well as increase the expression of vascular endothelial growth factor (VEGF) receptors and vascular endothelial (VE)-cadherin (77). Shear stress also increases the production of tissue plasminogen activator (7) and nitric oxide (NO) (7, 78) (two important molecules secreted by vascular ECs) in EPCs, indicating that EPCs can be a promising cell type for the construction of tissue-engineered vascular grafts. When subjected to shear stress, mouse ESC-derived Flk-1 positive (Flk-1$^+$) cells express EC markers such as Flk-1, Flt-1, VE-cadherin, and PECAM-1 (79). These Flk-1$^+$ cells do not upregulate smooth muscle (SM) alpha-actin, an early SMC marker, in response to shear stress, indicating that shear stress evokes the EC lineage as opposed to the SMC lineage. The cells experiencing shear stress also proliferate and form tube-like structures much faster than the cells cultured under static conditions.

Studies have shown that MSCs grown *in vitro* in the presence of different chemical factors can differentiate into vascular ECs or SMCs (80-84). However, limited research has focused on the effects of fluid shear stress on MSC differentiation into vascular cells. Fluid shear stress has been reported to increase the number of SM myosin heavy chain positive cells derived from rat marrow stromal cells. However, the amount of SM alpha-actin was not significantly different to static controls (85). Conversely, it has been shown that fluid shear stress promotes the expression of EC markers such as CD31 in cells derived from a mouse embryonic mesenchymal progenitor cell line. Interestingly, shear stress increases angiogenic VEGF gene expression while decreasing TGF-beta1 by approximately 50% at the transcriptional level (86). In our studies on adult human MSCs, we have also found that several angiogenic factors, including VEGF, fibroblast growth factor-1 (FGF-1), along with vascular SMC markers, including transgelin (SM-22alpha) and calponin, are significantly upregulated by shear stress (Hsu, unpublished data). However, unlike the previous study, we have shown that flow increases instead of decreases TGF-beta1. The difference in these TGF-beta1 trends could be attributed to the use of different cell lines, culture conditions, and shear stress levels used in each study. Our studies uncover a novel signaling mechanism in MSCs due to fluid shear stress activation of TGF-beta1/SMAD pathway. Overall, these shear stress studies show that stem cells and precursors are a potential cell source to promote vascular tissue remodeling.

There are also studies focusing on the possibility of shear stress producing a bone phenotype in MSCs. MSCs, when plated on bone-like ECM, increase calcium deposition in response to shear stress, (87, 88). Pulsating flow on adipogenic-derived MSCs cultured in osteogenic media increases NO production and COX-2 gene expression, similar to the response of bone cells; however, unlike bone cell response to flow, there is no increase in osteopontin or collagen type I alpha 1 (88). These data suggest that MSC response to shear stress is dependent on the chemical factors present in the microenvironment.

3.2. Effects of mechanical strain on mesenchymal stem cells

In addition to shear stress, the pulsatile nature of hemodynamic stresses results in cyclic tensile strain in cardiac tissue and the blood vessel wall. Mechanical strain is prevalent in numerous tissue types, and the exact mode in which it is applied is governed by several parameters. For example, physiological tensile strain may be cyclic in nature, as in the rhythmic distension of a blood vessel, or it may be fairly static, as in maintaining the extension of a bundle of skeletal muscle. Additionally, the applied load may occur in any number of directions within the 3-D

architecture of a given tissue, ranging from predominantly linear to more intricate multi-axial strains.

Although the mode of mechanical strain is often complex *in vivo*, its effects and underlying mechanisms can be investigated more readily with simplified *in vitro* models. In 2-D cell culture, equiaxial strain (i.e. equal strain in all directions) can be applied to adherent cells on a circular deformable substrate using negative vacuum pressure underneath the substrate. This type of *in vitro* stimulation is often created using a Flexercell mechanical cell culture system (Flexcell® International) or a similar custom-built device. A slight modification to this system employs a straight loading post placed directly under a portion of the deformable substrate such that its deformation is restricted to a single axis (89). This results in "uniaxial strain," which can alternatively be achieved by fastening a deformable substrate between two attachment points, one of which remains stationary while the other moves linearly to apply the strain (90). More complicated systems can be used to create varying degrees of anisotropic 2-D strain, such as an elliptical substrate deformed by vacuum pressure (91). While these models allow for investigation of simplified 2-D strain modes, more accurate representations of physiological forces may be modeled using 3-D cell culture constructs. These constructs consist of a deformable 3-D matrix seeded with cells. Some examples include a collagen ring seeded with SMCs to mimic a blood vessel (92) or a 3-D rectangular collagen lattice seeded with myoblasts to mimic skeletal muscle (93). Constructs such as these can be mechanically loaded through various methods ranging from simple linear stretch to computer-controlled multi-dimensional strain. The resulting strain delivered to cells may include a combination of translational, rotational, and/or multi-axial strains.

The *in vitro* response to mechanical strain often depends on several factors including the type, magnitude, frequency and duration of the strain, the matrix molecules used in culture, and the cell type being investigated. This array of factors has repeatedly led to seemingly conflicting reports in the scientific literature. However, comparing these studies in a previous review, we have found that the overall trend is that cyclic tensile strain promotes expression of various SM markers in SMCs and affects both SMC proliferation and matrix remodeling (94). Additionally, we reported that some studies have demonstrated similar effects of tensile strain on mesenchymal stem cells, thereby suggesting the potential use of mechanical stimuli for stem cell maintenance and differentiation. In this section, we will address mechanotransduction in cardiac muscle, as well as provide a more comprehensive overview of the effects of tensile strain on stem cells.

Similarly to vascular SMCs, cardiac muscle cells are constantly subjected to hemodynamic forces. In cardiac muscle, it is well established that this mechanical load is a major determinant in cardiac hypertrophy (increase in cell size). However, the mechanisms by which these forces are converted into intracellular signals are only beginning to be uncovered. Uniaxial "step" stretch of 20% has been found to induce hypertrophy in rat cardiac myocytes but induce hyperplasia (increase in cell number) in non-myocytes, suggesting that load-induced cardiac hypertrophy is a cell-type specific phenomenon (95). Additionally, this mechanical strain rapidly (within 30 – 60 min) induces several "immediate-early" genes known to be involved in the response to various growth stimuli, followed by expression of "fetal" genes such as skeletal alpha-actin, atrial natriuretic factor, and β-myosin heavy chain. These events closely resemble those of *in vivo* load-induced cardiac hypertrophy, which demonstrates that uniaxial step stretch may be a reasonable mechanical model for this phenomenon. In subsequent studies by the same group, it was found that cardiomyocytes must transduce the stretch signals through a mechanism other than electrical or direct cytoskeletal signals alone (96), rapidly activating a large number of second messengers, including tyrosine kinases, $p21^{ras}$, MAPKs, S6 kinases, protein kinase C, phospholipase C, and phospholipase D, but not cAMP (97). Additionally, stretch-conditioned media alone could similarly induce these events in non-stretched cardiomyocytes, suggesting the involvement of an autocrine or paracrine signaling mechanism. In conjunction with the previous studies, these reports suggest that tensile strain acts as a potent stimulant of cardiac muscle cells, and that this stimulus can lead to complex, yet predictable cell responses, including an initial early response, followed by progression of cell-signaling events, and eventually leading to an overall hypertrophic response.

It is important to note that while this strain-induced response in cardiac myocytes is reasonably well defined, it is considerably different from the response of vascular SMCs to tensile strain mentioned in our previous review (94). Not only does this suggest that tensile strain is important in regulating several different cell types, but it also suggests that seemingly similar mechanical stimuli may play various roles in directing cell and tissue development. This is particularly important when attempting to utilize mechanical stimulation to direct stem cell differentiation toward a particular lineage, as is being done in a number of tissue engineering laboratories. In terms of tensile strain for skeletal and vascular tissue engineering, we will focus on three main types of stem cells: skeletal muscle precursors (including both satellite cells and myoblasts), ESCs and MSCs.

While there exists some inconsistency in the field, it is generally agreed that satellite cells are adult muscle stem cells which are normally quiescent, but proliferate during muscle growth and regeneration after injury or disease, whereas myoblasts are early muscle precursors that either fuse to form myotubes, or differentiate into satellite cells (98, 99). Due to their potential to regenerate muscle tissue in a mechanically-stimulated environment, several studies have used tensile strain as a stimulus to invoke differentiation and proliferation of these cells. Rat satellite cells cultured under conditions of 25% equiaxial cyclic strain at a frequency of 5 cycles per minute (cpm) increase proliferation within as

little as 2 hours of stimulation (100), while in a more physiologically relevant model, 10% equiaxial strain activates satellite cells on a mechanically strained muscle fiber after only 30 minutes of strain (101). Cyclic strain induces the release of hepatocyte growth factor (HGF), which is necessary for the stretch-induced increase in proliferation (100). Strain also activates NO synthesis and thus MMP, leading to HGF release from matrix (102, 103). While these studies were designed specifically to provide insight into the molecular basis of the satellite cell strain-response, additional studies have been created to better mimic the *in vivo* mechanical stimulation of skeletal muscle precursors leading to myotube formation. Rat satellite cells in a 3-D environment subjected to 10% uniaxial cyclic strain align with the axis of stretch, become oblate in shape, and form myotubes, whereas control cells spread in all directions and do not display myotube formation. This demonstrates that mechanical stimulation of satellite cells within a 3-D construct may aid in cell alignment and differentiation (104). Likewise, myoblasts subjected to cyclic strain upregulate insulin-like growth factor-I (IGF-I), which initiates the fusion of myoblasts into myotubes. Interestingly, 1-hour ramp stretch upregulates mechano growth factor (MGF), a molecule involved in proliferation of myoblasts to establish the satellite cell pool (93). The authors hypothesized that this phenomenon may relate to the early and late stages of muscle development in which early spasmodic contractions of newly formed myotubes are similar to cyclic loading, while later developmental steps may include slow traction caused by bone growth similar to the ramp stretch regimen. Thus, early cyclic stimulation might aid in production of the satellite cell pool, and later ramp loading might aid in adult muscle development. This study effectively demonstrates that intricate 3-D models of mechanical stimulation can be used to mimic *in vivo* cell behavior, and that variations of a single mechanical stimulus may have diverse effects on a given cell type. When comparing equiaxial to uniaxial strain of C2C12 myoblasts in culture, both modes of strain increase ERK and Akt phosphorylation, but only equiaxial strain results in the phosphorylation of ribosomal S6 kinase, once again demonstrating the importance of strain mode on cellular response (105).

The lessons learned from these studies have significant implications for tensile loading of other stem cells as well, particularly in terms of choosing an appropriate mechanical loading system to produce the desired cell lineage. As seen in the previous section, both ESCs and MSCs have the potential to differentiate into vascular cell types in response to fluid shear stress for the use in a mechanically-loaded vascular tissue engineering construct. While the differentiation potential of ESCs vastly outweighs that of MSCs, MSCs are far easier to obtain (i.e. bone marrow extraction) and are currently less controversial for cellular therapies. 2-D cyclic uniaxial strain has been shown to be a potential stimulus for the differentiation of MSCs into a vascular SMC phenotype (90, 106). Bone marrow-derived progenitor cells subjected to 10% cyclic uniaxial strain at 60 cpm for 7 days proliferate less and express more SM markers SM alpha-actin and h1-calponin (106). Uniaxial cyclic strain also causes the cells to realign perpendicularly to the axis of strain, a phenomenon that had been previously reported in SMCs by Kanda (107). In our uniaxial strain studies, we also found an increase in SM markers (alpha-actin and SM22-alpha) in MSCs subjected to 5% strain at 60cpm, but the levels return to basal levels after the cells realign perpendicularly to the axis of strain. In contrast to uniaxial strain, we found that equiaxial strain decreases expression of both these markers, again displaying the importance of the type of strain for a particular response. Not only is the type of strain important, but also the orientation of the cells in regards to the axis of strain. To better control MSC alignment in the axis of strain, we investigated the use of micropatterning to maintain stem cells aligned with the axis of uniaxial cyclic strain. The model system in Figure 1 depicts how parallel microgrooves on the surface of an elastic membrane can orient MSCs through contact guidance to better mimic the uniaxial "circumferential" strain felt by SMCs in a blood vessel. In studies using cells seeded on these membranes, we found that cyclic strain with parallel-oriented microgrooves induces global changes in MSCs, including an increase in the SM marker calponin 1, decreases in cartilage matrix markers, alterations in cell signaling, and an increase in proliferation (108). However, when microgrooves are oriented perpendicularly to the axis of strain, some gene changes are diminished, and proliferation is unaffected. Our study suggests that mechanical strain regulates both differentiation and proliferation of MSCs, and that the effects of a given mechanical stimulus may be dependent on the orientation of cells with respect to the axis of strain. This phenomenon has considerable implications for vascular tissue engineering with MSCs as well as for mechanobiology of stem cells in general.

To direct ESC differentiation into various lineages, several groups have studied the effects of mechanical strain on ESC differentiation. Mouse ESCs in the 3-D environment of a microporous polyurethane tube were pre-incubated for 2 days with vascular VEGF followed by 2 days of pulsatile flow loading which included fluid shear stress on the luminal surface of the tube and circumferential tensile strain within the tube wall (109). In the absence of mechanical loading, mostly SM alpha-actin$^+$ cells were layered on the luminal surface of the tube. However, when pulsatile flow and circumferential strain were applied, the luminal layer differentiated to an EC phenotype, while the cells in the deeper layers of the tube wall displayed a SM phenotype, similar to the natural architecture of ECs and SMCs in a blood vessel. This study suggests that physiological mechanical stresses felt by each cell type (shear stress on ECs and circumferential strain SMCs) each promote the respective cell phenotype in MSCs. In addition to vascular cells, stem cells have been shown to differentiate into cardiac cells. Equiaxial cyclic strain (5-20%) on ESCs results in stimulation of angiogenesis as well as cardiomyogenesis based on visualization of PECAM-1$^+$ capillary areas and spontaneously contracting cardiac foci (110).

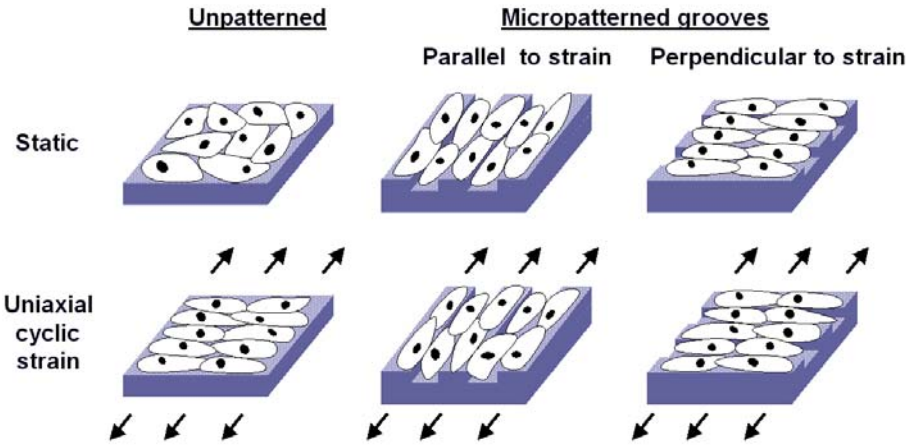

Figure 1. Different orientations and alignments of MSCs subjected to uniaxial strain. On an unpatterned surface, MSCs orient randomly during static conditions, but reorient perpendicularly to the strain axis during uniaxial cyclic strain. However, micropatterned grooves can be used to orient MSCs in a specific direction, ranging anywhere from parallel to perpendicular, with respect to the axis of strain.

As previously mentioned, tensile strain exists in many types of tissues, and this type of mechanical loading has implications for stem cell differentiation to other cell types as well. 2-D equiaxial cyclic strain at low strains has been shown to induce bone differentiation in MSCs for both human and rat, showing an upregulation of osteoblastic genes and matrix mineralization (111, 112). Under higher equiaxial strains, MSCs do not show an osteoblastic phenotype, again stressing the importance in the particular type of strain to induce a particular lineage (90, 113). A more complex mechanical regime involving concurrent cyclic axial and torsional strain applied to human MSCs in a 3-D matrix has been shown to result in differentiation towards a ligament phenotype (114). Even more intriguing is a study suggesting that mechanical stimulation might be used to prevent stem cell differentiation (115). The authors reported that with 2-D cyclic equiaxial strain of 10% or greater (frequency of 6, 10, or 30 cpm), hESCs exhibit reduced differentiation and increased self-renewal.

While these results may seem confusing, they are not necessarily implausible. As mentioned previously, the sheer number of adjustable parameters in these investigations can result in seemingly similar studies with completely different results. The important point however, is that these studies demonstrate the vast possibilities for mechanical stimulation of stem cells as well as the need for better control of the parameters involved.

3.4. Effects of the rigidity of extracellular matrix on mesenchymal stem cells

Cells are not only sensitive to dynamic mechanical stimuli but also sensitive to the static mechanical environment, such as the stiffness of their ECM. The range of stiffness in the body is enormous, from soft, pliable brain tissue with Young's modulus of tenths of a kilopascal (kPa) to hard, calcified bone with a modulus of hundreds of kPa (Figure 2). With three orders of magnitude separating the softest and stiffest tissue in the body, these tissues contain cells that are tuned to the specific mechanical environments in which they reside. Focal adhesions and transmembrane integrins serve as lines of communication from the ECM to the cytoskeleton, which reorganizes based on the traction forces the cell exerts on the matrix. A delicate force balance is created, with parameters that depend on the rigidity of the matrix, activation of actomyosin motors and the resulting tension in the cytoskeleton (116). This reorganization of the cytoskeleton that molds the cell shape can have a huge impact in cellular pathways that regulate migration, proliferation and differentiation.

Pelham and Wang developed a controlled system using polyacrylamide gels with varying acrylamide and bis crosslinker concentrations in order to carefully control substrate rigidity to study its effects on fibroblast migration (117). Keeping the chemical composition of the substrate the same, they found that the rigidity of the substrate alone is responsible for morphological, protein and motility changes. Cells grown on a softer substrate spread out less, have more dynamic but irregularly shaped focal adhesions, express less phosphotyrosine and migrate at faster rates. The decrease of tyrosine phosphorylation in fibroblasts grown on soft surfaces may be responsible for less stable focal adhesions, accounting for these morphology and motility changes. Although fibroblasts migrate faster on softer substrates, in terms of directionality fibroblasts prefer to migrate toward a rigid surface (30kPa) from a softer one (14kPa), but from a rigid surface, cells turn around once they reach the soft surface (118). This directionality in response to the rigidity, or durotaxis, can be explained by the increase in traction forces on rigid surfaces pulling the region forward, which depends on FAK to sense the matrix rigidity (119). The decrease in FAK and other focal adhesion proteins on soft substrates in fibroblasts is mediated by

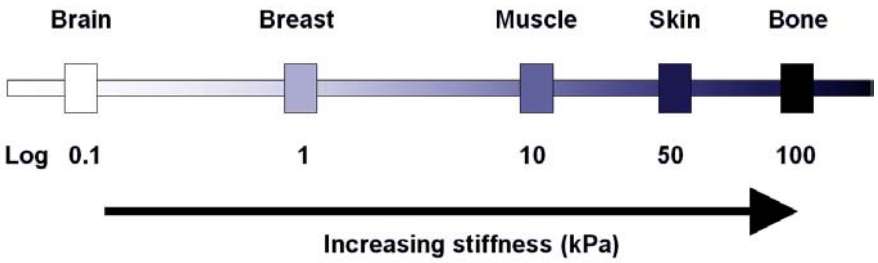

Figure 2. Young's modului of tissues covers a range from 0.1-100kPa. Brain tissue is the softest, while bone is the hardest. When grown on different stiffness, cells *in vitro* respond differently depending on the cell type in regards to proliferation, migration and differentiation.

the $alpha_2beta_1$ integrin, which when blocked in fibroblasts, abolishes the decrease in FAK on soft surfaces (120). Along with the $alpha_2beta_1$ integrin , $alpha_vbeta_3$ and $alpha_5beta_1$ integrins have also been shown to be involved in rigidity sensing in fibroblast migration on fibronectin-coated surfaces (121, 122).

Just as studying fibroblast migration on different rigidities is important for wound healing applications, studying SMC migration on different rigidities is important in terms of cardiovascular applications. Restenosis, the renarrowing of a blood vessel largely due to SMC migration from the media to the lumen of the blood vessel, is one situation in which it is critical to understand SMC migration. Like fibroblasts, SMCs migrate faster on softer gels (15kPa) compared to stiffer gels (28kPa), and exhibit the same trend in durotaxis, moving toward a more rigid surface from a soft surface (123). SMC spreading is also increased on a rigid surface, spreading even more with higher collagen density for rigid surfaces but only minimally so for soft surfaces (124). Contrary to fibroblasts, however, SMCs display a biphasic response in migration speed on a range of rigidities (1-308kPa) (125). This peak response in migration shifts, depending on the chemical composition of the substrate. Peak migration rates occur on a stiff surface (51.9kPa) with low fibronectin density ($0.8\mu g/cm^2$) or a softer surface (21.6kPa) with high fibronectin density ($8\mu g/cm^2$). Clearly, both the chemical and mechanical factors of the ECM interact with each other, affecting migration rates on different rigidities. The influence on migration rates is found to be mediated by the Rho/Rho-kinase (ROCK) pathway. This pathway regulates the amount of polymerized actin in the cell, affecting cell shape and in turn other cell functions such as migration and differentiation (126). Our group has also found that Rho and ROCK are involved in 3-D migration of SMCs on soft collagen gels (127). The differences in focal adhesion and actin stress fiber formation on different rigidities are responsible for the differences in cell spreading and migration in SMCs.

Similarly, tumor cells migrate faster on softer surfaces in 3-D matrix (128). However, cancer cells behave differently from normal cells in matrix rigidity-dependent proliferation, possessing the anchorage-independent ability of living in a soft matrix that may induce apoptosis in normal cells (129). The ability of cancer cells to grow on soft agar suggests that they have lost the ability to respond to matrix rigidity compared to normal cells. Tumors, which are stiffer than normal tissue, modulate changes in cells leading to enhanced proliferation due to increases in Rho-generated cytoskeletal tension and ERK activation (130). Understanding these mechanisms is important in order to eventually control the proliferation and migration of the cells that thrive in tumors.

Even normal cells that reside within the same organ can respond differently to rigidity. Although both residing in soft brain tissue, astrocytes and cortical neurons respond in different ways when grown on soft substrates (131). Astrocytes exhibit limited spreading and disorganized actin filaments on soft substrates whereas cortical neurons extend neurites and polymerize actin filaments on both hard and soft surfaces. On soft surfaces with rigidity similar to brain tissue, neuron growth dominates over astrocyte growth while on hard surfaces astrocyte growth is preferred. Clearly the physical nature of the substrate is important in signaling to particular cell types how to migrate as well as proliferate. A cell's ability to sense matrix rigidity can be crucial since different cell types need to respond to mechanical cues differently in order to orchestrate their functions correctly.

Besides altering cell morphology, migration, and proliferation, matrix rigidity can lead to a complete reprogramming of cell functions. The rigidity of the matrix has been shown to be important in differentiating precursor cells for a variety of cell types. For example, breast epithelial cells cultured in floating 3-D collagen gels but not attached collagen gels, differentiate into tubules, which is regulated by ROCK-mediated contractility and a subsequent down-regulation of Rho and FAK function (132). In myogenesis, rigidity is important for myotube striation (133). Cells only striate when grown on gels of similar stiffness as muscle (12kPa), and do notstriate on much more rigid or much softer surfaces. In another example that illustrates the importance of native tissue stiffness, pre-osteoblastic cells deposit less mineral on soft surfaces compared to rigid surfaces that are more like the rigidity of bone matrix (134). On soft surfaces with low collagen density, these cells proliferate half as fast, migrate slower, and have poorer actin cytoskeleton organization and immature focal adhesions compared to rigid surfaces. The common theme that presents itself is that cells behave

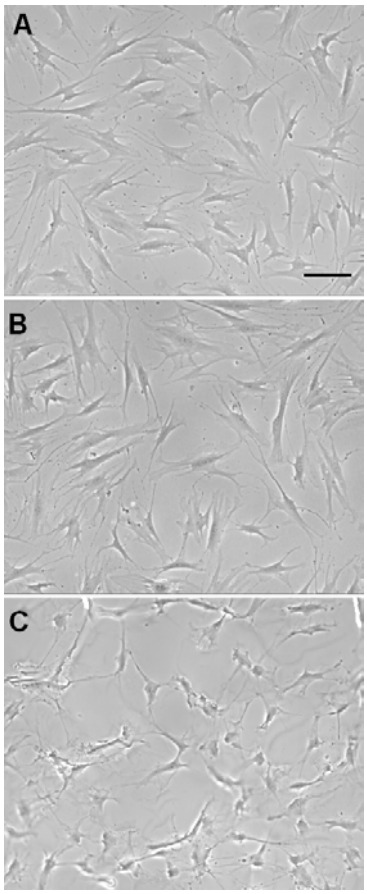

Figure 3. MSCs grown on varying rigidity surfaces. MSCs were seeded on collagen-coated glass (A), 15kPa polyacrylamide gel (B) and 1kPa polyacrylamide gel (C). Cells display a distinctly different morphology on a softer matrix compared to a rigid one. Bar = 100μm.

how they are "supposed" to behave when grown in an environment similar to their native environment. As mounting research suggests, not only is the chemical milieu a critical factor, but the physical nature of the substrate is just as important to mimic.

In addition to differentiating precursor cells into their respective phenotypes, matrix rigidity can also have profound effects on MSC differentiation. MSCs differentiate toward a bone, muscle, or neuronal phenotype when grown on hard, medium or soft surfaces that are akin to the stiffness of their respective tissue (135). The expression of these differentiation markers is more pronounced when cells are grown on their "native" rigidities in addition to treatment with chemical factors known to promote the particular phenotype, compared to cells grown in chemical factors alone. The interaction of chemical and physical factors can have a synergistic effect, one factor needing the other to stimulate certain signaling pathways. In studying the SM phenotype, we have previously reported that the chemical factor TGF-beta1 promotes higher expression of SM markers in MSCs on polystyrene (136). With decreasing rigidity, we have found that MSCs display less SM markers, and the TGF-beta1-stimulated increase on rigid surfaces does not occur on soft surfaces (Park, unpublished data). Since TGF-beta1 is important in both smooth muscle and cartilage differentiation, matrix rigidity may be the defining stimulus that signals MSCs to follow either lineage. MSCs grown on different rigidity surfaces are shown in Figure 3.

Although many studies have been performed by isolating the physical properties of the substrate to study their effects on cells, much needs to be done in understanding the underlying mechanisms and functional consequences. Studying these processes on different rigidities is helpful in understanding stem cell differentiation that occurs in the native tissue environment (as opposed to *in vitro* experiments commonly performed on polystyrene), as well as to study cell behavior in disease and healing processes such as fibroblast migration in scar tissue, SMC proliferation and migration in atherosclerosis and restenosis, rigidity-independent cancer cell growth and tumor formation, myocardial infarction (MI) in which stiff scar tissue prevents remodeling (137), neurodegenerative disease in which neural cells cannot cross rigid scar tissue and numerous other afflictions. Finally, future studies should focus on studying cells in a 3-D environment to better mimic *in vivo* conditions since there are clear differences in the way cells behave in 2-D vs. 3-D (127, 128).

4. USING MESENCHYMAL STEM CELLS FOR CARDIOVASCULAR THERAPIES

4.1. Biomaterials for cardiovascular tissue engineering

Biocompatible matrix materials are an important element of cardiovascular tissue engineering. They provide a temporary scaffold for the attachment, migration, and survival of transplanted cells as well as native cells (138, 139). The ideal scaffold would support a three-dimensionally linked porous structure for cell migration and proliferation, biodegrade at a rate that matches the rate of cellular in-growth and matrix secretion, integrate over time resulting in neo-tissue that is morphologically and functionally similar to native tissue, enable cell attachment and viability, and provide mechanical strength that matches that of the *in vivo* environment (140). Among the various types of polymer scaffolds, naturally-derived and synthetic polymers are the two major categories of matrix materials.

4.1.1. Naturally-derived polymers

Stem cells have been cultured and differentiated on a variety of naturally derived or synthetic biomaterials. Some native matrix materials are collagen, laminin, fibronectin, matrigel, hyaluronic acid (HA), and fibrin. These native matrix materials can be purified from donor tissues to support cellular expansion and differentiation *in vitro* and *in vivo*. Among these native biopolymers, collagen, fibrin, matrigel, and HA have been most widely used to reconstitute the 3-D ECM environment.

The collagen family contains approximately 20 types of triple helical fibrous proteins, and constitutes about one third of the total proteins in our body (141). Collagen supports cell adhesion, provides structural integrity, and bears mechanical loads (142). Synthesized in a cell in procollagen form, extracellular proteolytic enzymes cleave the molecules of procollagen into collagen, which then assemble into fibrils (143). Collagen can be degraded by the enzymatic activities of acid phosphatase, leucine amino peptidase, collagenase, and MMPs (144, 145). Among the types of collagen, the type I form is prevalent in a wide range of tissues such as cardiac tissue, blood vessels, bone, skin, and internal organs, and is widely studied *in vitro* as a substrate that promotes cell growth and differentiation via binding of $alpha_1beta_1$ and $alpha_2beta_1$ integrins.

Another widely studied ECM is fibrin, an FDA-approved biomaterial that is commercially available as a biological sealant and adhesive. Fibrin is a bioresorbable matrix that acts as a provisional material for cellular ingrowth during wound repair. Formed by polymerization of fibrin monomers, it is resorbed by degradation of fibrinolytic enzymes such as plasmin (146). Fibrin contains numerous angiogenic factors as well as the arginine-glycine-asparagine (RGD) motif that binds to cell receptors (147).

Matrigel is a murine basement membrane matrix that is extracted from Engelbreth-Holm-Swarm sarcoma. Matrigel is rich in laminin, fibronectin, collagen IV, proteoglycans and other bioactive factors in the matrix (148). In addition, Matrigel also contains growth factors such as bFGF, IGF-1, and TGF-beta. The effect of Matrigel on stem cells is unclear, but it is believed that stem cells respond to angiogenic and growth-promoting signals found in the matrix (149). It is likely that cell-cell contacts formed in the 3-D Matrigel environment also play a role in stem cell differentiation (150).

HA is a viscoelastic carbohydrate that is composed of disaccharide unit repeats of glucuronic acid and N-acetyl glucosamine (151). Found in the ECM and in various organs, HA is synthesized by one of three hyaluronic acid synthases on the plasma membrane and becomes degraded by hyaluronidases (152). HA plays numerous roles in the body, such as resisting compressive forces between joints and creating space fillers for cellular ingrowth (143). HA also modulates cell adhesion, motility, and proliferation during tumor formation and wound healing (153, 154).

4.1.2. Synthetic polymers

Unlike natural biomaterials, synthetic biopolymers are produced from chemical synthesis. Already approved for use in various medical applications such as sutures, synthetic polymers are favorable due to the ease of controlling their chemical, structural, and mechanical properties. Furthermore, they can be fabricated as porous 3-D structures by particulate leaching, high pressure processing, freeze-drying, electrospinning, and other processes (155-158). The macroscopic structure can be shaped into tubular conduits or heart valves by post-processing extrusion, injection molding, or 3-D printing methods (140, 159-161).

The most commonly studied polymers are aliphatic polyesters, namely poly-L-lactic acid (PLLA) and polyglycolic acid (PGA), whose monomer subunits can copolymerize to form copolymers such as poly(lactide-*co*-glycolide) (PLGA). These polyesters degrade by hydrolysis when the hydrated areas of the polymer cleave ester bonds, reducing the molecular weight of the polymer in a continuous fashion. The mechanical properties become compromised until the polymer loses its integrity, at which point the cleaved molecules are carried away by the aqueous environment (162). The rate of biodegradation depends on polymer properties, including molecular weight and polymer ratio, as well as environmental factors such as the ionic environment and pH (163). Recent research to engineer synthetic polymers with improved elastic properties and resistance to plastic deformation led to the development of novel materials such as poly(glycerol-sebacate) and poly(diol citrates) (164, 165). In addition, self-assembling peptide nanofibers can provide microenvironments that are suitable for cell survival and migration (166, 167). For example, self-assembling RAD16-II peptide nanofibers injected into ischemic myocardium can promote angiogenesis by recruiting vascular ECs and SMCs, followed by the infiltration of cardiomyocytes. Further examples of polymers promoting cardiac function and neovascularization of the myocardium are discussed in the next section.

4.2. Mesenchymal stem cells for cardiac repair

MI is a major cause of mortality in the United States (168). It results from occlusion of coronary arteries supplying blood to the heart, leading to an insufficient supply of blood and oxygen to the heart. The pathological response to MI of the left ventricle (LV) includes the death of non-regenerative cardiomyocytes, thinning of the LV muscular walls, aneurismal thinning of the LV cavity, reduced cardiac output, and ultimately congestive heart failure (169, 170). Currently, the most effective treatment for heart failure is a heart transplant, but due to the shortage of available organs, alternative treatments are necessary.

A widely used therapeutic strategy for cardiac repair is based on the classical triad of important components in engineering tissues: the cell source, tissue inducing substance, and matrix (171). MSCs have shown tremendous promise as a cell source for cardiac repair. Current therapies include the delivery of MSCs alone or in conjunction with biocompatible matrix materials and growth factors that stimulate cardiac regeneration and neovascularization in the infarct zone. The engineered constructs can be pre-formed *in vitro* before implantation or delivered *in vivo* for *in situ* cardiac regeneration. Here we will discuss the therapeutic potential of delivering MSCs alone or in conjunction with biocompatible matrices for repair of MI.

Figure 4. Schematic of therapeutic cell injections into the heart for repair after MI. Therapeutic combinations may include MSCs, growth factors, and biocompatible polymers. Upon loading into the syringe, the treatment can be conveniently injected into the heart.

Therapeutic transplantation by injection is a promising mode of delivery because it requires only a minimally invasive surgical procedure. Using this method, cells, matrices, growth factors, and their combinations can be injected intramuscularly in or near the infarct zone (Figure 4). Using injectable delivery, MSCs have shown promising therapeutic results *in vivo*. In a swine model of autologous MSC implantation, MSCs engraft in the myocardium and express muscle-specific proteins as early as two weeks after implantation (172). These proteins include alpha-actinin, troponin-T, tropomyosin, and myosin heavy chain, showing myogenic differentiation had occurred. In addition, the degree of cardiac dysfunction is significantly attenuated after four weeks along with a reduction in infarct wall thinning. Besides myogenic differentiation, another proposed mechanism of involvement for MSCs in the infarct zone is paracrine stimulation. After injecting autologous MSCs into a rat peri-infarct zone, Tang reported that the protein expression of angiogenic growth factors bFGF and VEGF is enhanced in the MSC-treated animals, compared to the animals with control media treatment (173). The enhancement of angiogenic proteins is accompanied by an increase in stromal cell-derived factor-1alpha, a stem cell homing factor, as well as downregulation of pro-apoptotic protein Bax. In addition, fractional shortening, which is a measurement of cardiac performance, is significantly improved in MSC-treated hearts. The MSC-treated animals also have enhanced capillary density as a measure of neovascularization. Based on these results, MSCs appear to enhance paracrine stimulation of angiogenic and pro-survival growth factors, while enhancing cardiac function and neovascularization. Together, these results demonstrate that injections of MSCs can significantly attenuate the pathological remodeling process associated with MI, enhance cardiac function, and promote neovascularization.

Recent studies have examined whether genetically modified MSCs can show further enhanced therapeutic effects. Rat MSCs transfected with human $VEGF_{165}$ demonstrate the most improvement in infarct size, infarct wall thickness, and capillary density, in comparison to control animals that receive LacZ-transfected MSCs or media injections (174). In addition, cardiac function, as assessed by ejection fraction measurements, is highest in the VEGF-overexpressing MSCs group. Another study that investigated the therapeutic effects of rat MSCs genetically modified with Akt1 pro-survival gene show that these MSCs inhibit pathological remodeling by reducing the intramyocardial inflammation and regenerate about 80% of the lost myocardial volume. (17). Cardiac function is completely normalized after treatment with the genetically modified cells. Together, these results suggest that the combined treatment of cells with gene therapy could have enhanced therapeutic outcomes.

Besides genetic modification of cells, MSC injections with polymers have also shown promising results. Our results show that combined delivery of human MSCs and fibrin in a chronic MI model in *rnu* nude rats lead to enhancement in neovascularization, as assessed by microvascular density, in comparison to saline alone and fibrin alone control groups (Huang, unpublished data). However, treatment of cells alone, fibrin alone, and the combination of cells and fibrin all attenuate dilatation of the LV cavity. *In vitro* characterization of gene expression of MSCs in the presence or absence of fibrin show over a 15-fold increase in angiogenic growth factor PDGF-B and survival-related gene HSP70 in MSCs in the fibrin group. The gene expression analysis suggests that fibrin could activate angiogenic and pro-survival genes in MSCs. Together, our results implicate the delivery of MSCs with fibrin as a potent treatment for repair after chronic MI. Using a related cell type, Ryu tested the feasibility of delivering bone marrow mononuclear cells (BMMNCs) with fibrin in a rat MI model (175). Their results also corroborate our findings with MSCs in that implantation of BMMNCs with fibrin leads to more extensive neovascularization, compared to cells without matrix. We have also previously shown the improvement of cardiac function and angiogenesis by delivery of fibrin with myoblasts (176, 177).

Another approach for cardiac regeneration involves engineering cardiac tissue constructs *in vitro* before *in vivo* implantation. The advantage of this approach is that the cellular, structural, and chemical properties of the construct could be tailored prior to *in vivo* transplantation. Early studies demonstrated the proof-of-concept of constructing cardiac patches using dissociated

cells from the heart. In one study, circular rings of engineered heart tissue (EHT) made of rat cardiomyocytes were embedded in collagen I gel and matrix factors (178). When the construct was subjected to mechanical stretch, the ring-shaped structure developed interconnected and longitudinally oriented cardiac muscle bundles that are characterized by highly organized sarcomeric structure. The contractile properties of the EHTs were assessed to be similar to that of native myocardium. The authors then examined the suitability of the EHTs for MI repair by implanting five fused EHTs to the epicardium of the infarct zone on immune-suppressed rats (179). Their results show that the heart tissue is electrically coupled to the native myocardium and do not induce arrthythmias. More importantly, the EHT prevents dilatation of the myocardium, induces wall thickening, and improves fractional shortening. This study highlights the feasibility of engineering heart constructs that could support contractile function and improve cardiac function of infarcted hearts. Recently, transplanted monolayered sheets of MSCs onto the scarred myocardium after MI have been found to form a thick stratum of neovessels, undifferentiated cells, and few cardiomyocytes (180). The MSC graft reversed wall thinning in the scar area and enhanced cardiac function. This study demonstrates a new strategy for cardiac tissue engineering using MSCs.

In summary, MSCs demonstrate tremendous potential for repair of MI, whether by direct injection or by implantation of pre-formed cardiac constructs. The ability of MSCs to differentiate into cardiovascular cell types, improve cardiac function, and stimulate angiogenesis, all make MSCs an attractive cell source for cardiac repair.

4.3. Mesenchymal stem cells for vascular construction

Over 500,000 coronary bypass graft procedures are performed in the United States each year. The most commonly used bypass graft is the saphenous vein graft. However, the use of vein grafts is limited by the availability and 50% ten-year failure rate (181). Although synthetic vascular grafts composed of such nondegradabe materials as poly(ethylene terephthalate) (Dacron™), expanded poly(tetrafluoroethylene) and polyurethane have been successfully used to graft large blood vessels (182, 183), their performance is severely diminished in vessels smaller than 5mm in diameter (184). The major causes of failure include thrombogenicity and intimal hyperplasia from compliance mismatches. Although coating the luminal surfaces of these grafts with ECs reduces their thrombogenicity, the effect is short-lived (185). Moreover, nondegradable synthetic grafts do not allow for cellular remodeling or vasoreactivity. Tissue engineering of small blood vessels is a promising approach for fabricating biological vascular substitutes that are non-thrombogenic, fully functional and mechanically and morphologically similar to native blood vessels. Native blood vessels include three distinct layers: (1) EC monolayer as the inner lining, (2) media layer that is mainly constituted of collagen and elastin matrix populated by SMCs, and (3) adventitia layer (outer layer) that is constituted of connective tissue and fibroblasts.

The general vascular tissue engineering strategy combines a cell source and a suitable extracellular matrix to provide mechanical and structural support. In order to improve the patency and functionality of vascular grafts, tissue engineering strategies have focused on biological components or biodegradable polymers as graft materials. The first reported biological model of an artery was fabricated by seeding ECs and SMCs in a collagen gel tube (186). In this study, the endothelial layer functioned normally, producing von Willebrand factor (vWF) and prostacyclin, however the strength of the vessel depended on the combination of collagen combined with a Dacron™ mesh. Although improvements have been made, using mechanical conditioning (187) or glycation of the matrix to increase strength (188), collagen gel based scaffolds are still too weak to withstand high arterial pressures. Other biopolymers such as fibrin have shown some promise for vascular tissue engineering, with higher mechanical strengths than collagen. Fibrin-based tubes seeded with SMCs and ECs remained patent for 15 weeks in lamb models and demonstrated significant matrix remodeling and vasoactivity (189). These grafts also had mechanical properties closer to native vessels than collagen-based grafts have shown. Hyaluronan-based scaffolds have also shown promise, with grafts implanted in the abdominal aortas of rats exhibiting long-term patency, recruiting EC and SMC layers and fully degrading after 4 months. (190). Rigorous mechanical studies were not performed, but *in vivo* studies showed that the grafts were able to withstand blood pressure.

Decellularized tissues also hold promise as non-thrombogenic compliant grafts that are amenable to cellular remodeling. The main advantage to these grafts is that the ECM with all its different components is completely formed, and does not need to be fully created by cells. This leads to a shorter preparation time for these grafts since they are initially stronger compared to collagen and fibrin grafts alone, whose starting materials have been reconstituted and are no longer in their original crosslinked forms, relying on secretion of matrix and enzymes by the cells which takes significantly longer to produce. Vascular grafts from decellularized tissues have shown excellent hemostasis and patency within three months (191) and can also be reseeded with vascular cells *in vitro* (192). On the other side of the spectrum of decellularized tissues is starting from cells only, without any matrix scaffold. For example, vascular grafts can be successfully generated *in vitro* exclusively from sheets of SMCs and fibroblasts, called “sheet-based tissue engineering” (193, 194). These grafts can then be seeded with ECs, implanted in various animal models and shown to be patent and mechanically sound for 8 months *in vivo*. However, the long fabrication time (8 weeks) and autologous nature of the cells associated with such an approach make it impractical for emergency clinical needs. Although various cell types have been studied for vascular engineering applications, an optimal source has yet to be agreed upon. Aside from the difficulty associated with isolating autologous SMCs and ECs from patients, the use of autologous SMCs is clinically impractical due to the effects of age on SMC proliferation and matrix synthesis (195, 196).

In addition to native biological materials, biodegradable synthetic polymers have also been widely studied as vascular graft materials. The majority of the focus has been on the biodegradable polyesters because they are biocompatible, FDA approved, and highly customizable. Their degradation rate and mechanical properties can be fine tuned by simply varying the types and ratios of the starting monomers. ECs and SMCs cultured in a tubular PGA porous scaffold under pulsatile flow conditions were implanted *in vivo*, and remained patent for 24 days (197). The scaffold developed vascular histological features that were consistent with vascular structures. A vascular graft composed of a PGA sheet and poly(lactide-co-capralactone) copolymer seeded with fibroblasts and SMCs was implanted in dogs and showed similar appearance to native vessels after 6 months, with no thrombus, stenosis or dilatation (198). Although these PGA studies have shown promising results, there is evidence that products from PGA hydrolysis lead to SMC dedifferentiation (199).

The micro and nano structure of the scaffolds can also play a role in the success of vascular grafts. With the use of electrospinning technology, scaffolds with nanoscale features that closely mimic native fibrous ECM can be fabricated from both synthetic and biological polymers (200, 201). These scaffolds are composed of nonwoven nanoscale fibers and exhibit large surface areas and high porosity, potentially allowing more efficient cell infiltration and remodeling. Evaluation of nanofiber scaffolds for vascular tissue engineering has only recently begun. A collagen blended poly(l-lactide-co-capralactone) nanofiber scaffold demonstrated successful EC attachment and viability *in vitro* (202). Nanofiber scaffolds can also be aligned and layered to mimic the ECM and cellular organization of native blood vessels (203). Shear stress-induced orientation of the EC layer observed *in vivo* can be mimicked *in vitro* by seeding ECs on oriented nanofiber scaffolds (204). Similarly, SMCs cultured on aligned nanofibers oriented their cytoskeleton and alpha-actin networks parallel to the fiber orientation.

Besides the matrix, an appropriate cell source is also crucial to a successful tissue engineered vascular graft. MSCs could potentially be an ideal cell source for vascular tissue engineering grafts. MSCs can be isolated and easily expanded *in vitro*. Their ability to differentiate into both vascular SMCs and ECs has already been demonstrated. Moreover, their ability to modulate the body's immune response could make it possible for allogenic implantation of MSCs and the fabrication of off-the-shelf vascular grafts. In our vascular graft studies, we have used PLLA nanofibers to fabricate small diameter (0.75 mm) vascular grafts integrated with human adult MSCs. Our *in vivo* rat studies suggest that MSCs create a non-thrombogenic surface, reduce platelet aggregation, and are capable of supporting endothelialization (Hashi, unpublished data).

Another potential source for autologous ECs is EPCs found in the bone marrow and in peripheral and cord blood. EPCs have been shown to have a highly proliferative and anti-thrombongenic potential comparable to ECs (205). EPCs seeded on to polymer scaffolds show signs of differentiation to ECs and improve *in vivo* patency rates (6, 7). Umbilical cord blood-derived human EPCs seeded onto 3-D porous PGA-poly(4-hydroxybutyric) acid or polyurethane tubular scaffolds in a biomimetic flow system express endothelial markers Flk-1, vWF, CD31, and CD34 and can functionally uptake acetylated low density lipoprotein (6). EPCs seeded on decellularized porcine scaffolds and implanted in sheep remained patent for 130 days and exhibited vasoactivity, while control grafts not seeded with EPCs occluded within 15 days (8). Stem cell-seeded vascular grafts are a promising new area of vascular tissue engineering. Many more studies need to be performed in order to confirm stem cell differentiation, recruitment of the proper cells and minimal immune response.

5. CONCLUSIONS AND PERSPECTIVES

MSCs, along with the other types of stem cells and progenitor cells outlined in this review, can have a huge potential for therapeutic use in cardiovascular applications, with promising therapeutic results outlined in Section 4. *In vivo* animal studies have shown promising results in both cardiac and vascular therapies, with MSCs promoting remodeling and vascularization, and even differentiating into the necessary tissue type. Before we can use MSCs routinely in human therapies, we must first thoroughly understand their response to both mechanical and chemical factors. Along with the many studies reviewed in Section 3, we have shown that mechanical factors induce important MSC changes in morphology, proliferation, gene and protein expression, and eventually differentiation. The signaling pathways that regulate these changes still need to be investigated. Our mechanobiology MSC studies have found that shear stress increases the expression of angiogenic factors. Uniaxial strain studies have also showed an upregulation in SM markers with physiological arterial strains, while equiaxial strain decreases SM marker expression, confirming that MSCs are indeed mechanosensitive and can distinguish between different types of strains. Lastly, SM marker expression in MSCs appears to be rigidity-dependent, with cells on soft surfaces expressing different cell type markers than those on rigid surfaces. In addition to rigidity, 3-D studies that closer resemble tissue architecture are more relevant to studying MSC response. Once these responses to chemical and mechanical stimuli are better understood, MSCs can become the ideal cell source for tissue engineering cardiovascular applications

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