Abstract—Mechanical forces have long been known to play a role in the maintenance of vascular homeostasis in the mature animal and in developmental regulation in the fetus. More recently, it has been shown that stem cells play a role in vascular repair and remodeling in response to biomechanical stress. Laminar shear stress can directly activate growth factor receptors on stem/progenitor cells, initiating signaling pathways leading toward endothelial cell differentiation. Cyclic strain can stimulate stem cell differentiation toward smooth muscle lineages through different mechanisms. In vivo, blood flow in the coronary artery is significantly altered after stenting, leading to changes in biomechanical forces on the vessel wall. This disruption may activate stem cell differentiation into a variety of cells and cause delayed re-endothelialization. Based on progress in the research field, the present review aims to explore the role of mechanical forces in stem cell differentiation both in vivo and in vitro and to examine what this means for the application of stem cells in the clinic, in tissue engineering, and for the management of aberrant stem cell contribution to disease. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: adventitia ■ atherosclerosis ■ stem cells

The term stem cell was initially used to describe a distinct embryoid cell with the capability to differentiate into any more specialized cell. Since the discovery that white blood cells were derived from common hematopoietic precursors, the usage of the term has expanded to include cells capable of infinite self-renewal and differentiation into any tissue at any stage of development. In recent years it has become increasingly apparent that such cells are present in almost all tissues throughout the body, although whether they have an active functional role in many cases remains unclear. The related term progenitor cell is usually used to describe a cell capable of differentiation into a small number of specific cell types with the capacity for self-renewal, albeit over a more limited time span. Embryonic stem cells (ESCs), adult stem cells, and vascular progenitor cells have all been put forward as potentially useful candidates for vascular repair and generation of tissue engineered vessels for patients with heart disease. It is possible to culture vascular cells directly from vessel samples, but stem/progenitor cells have the advantage of rapid replication and population expansion, allowing for the culture of the large number of cells that may be required for transplant, as well as a decreased immunogenicity. To generate healthy tissues and to ensure a strong commitment of cells to vascular lineages, it has been suggested that the differentiation process may benefit from replication of the different physical forces generated by blood flow during the cardiac cycle. These forces include shear stress, the friction exerted by the blood flow in parallel to the cell surface, and cyclic strain, the stretch as the blood vessel dilates in response to increased flow. These forces may also be involved in the differentiation of in situ progenitor cells, which may contribute to repairing the injured vascular wall, but could also become dysregulated in disease states and contribute to vessel remodeling. Shear stress has been studied extensively in relation to changes in endothelial homeostasis. Endothelial dysfunction is an early hallmark of vascular disease, including atherosclerosis, the development of which is thought to be related to reduced levels of shear stress. The role of cyclic strain is not as well characterized but is thought to play a role in the maintenance of smooth muscle cell integrity. An increased understanding of the role that physical forces have in shaping vascular lineage commitment will aid in harnessing the therapeutic potential of these cells.

Stem Cells With Potential for Vascular Repair

Embryonic Stem Cells

ESCs, pluripotent cells derived from the fetal blastula, showed early promise for potential therapeutic use and study of vessel development, because of their comparatively easy isolation from embryos and their rapid expansion. Because these cells can potentially commit to any cell line in order for them to be used therapeutically and to increase our understanding of vascular development, it is important that we elucidate the pathways through which they are primed for differentiation into vascular cell lineages in response to blood flow (Figure 1).
potential for tripartite differentiation into the mesodermal lin-
ential progenitors, expressing endothelial but not hematopoietic
mononuclear cells and possess characteristics of true endothe-
ing cells). These cells can be isolated from peripheral blood
cells with colony-forming ability (endothelial colony-form-
vascular regeneration, is a rare population of endothelial
Because these cells can be isolated from a relatively small volume of blood, their isolation also pres-
ents an opportunity to study a patient-specific population of endothelial cells minimally invasively. Their origin remains
eusive, however, partially because of the lack of consensus regarding their isolation and culture. It is proposed that they are either bone marrow–derived or migratory cells from the
Vascular lineage differentiation from embryonic stem
cells. Embryonic stem cells cultured in extracellular matrix
protein–coated plates activate integrins resulting differentiation
of Flk-1–expressing cells. These cells can further differentiate
into vascular precursors in response to mechanical stimuli. Shear
stress and cyclic strain can directly stimulate the progenitors
to differentiate into endothelial or smooth muscle cells, respectively.

Circulating Stem/Progenitor Cells
It was initially thought that stem cells were only active during early development. In recent years, however, it has become
increasingly clear that there exist a great number of cells in
adult tissues with the ability to differentiate into different cell
types when exposed to a particular stimulus within a specific
environment, for example, laminar blood flow against the
shear stress and cyclic strain can directly stimulate the progenitors
to differentiate into endothelial or smooth muscle cells, respectively.

Vascular Wall Resident Stem/Progenitor Cells
It is well known that endothelial cell turnover in the vessel wall
is uneven, that is, faster in the branching area where disturbed
blood flow occurs. Dead endothelial cells can be replaced by
replication of mature endothelial cells. However, recent evi-
dence supports the role of stem/progenitor cells in repairing
damaged vessels. It has been demonstrated that intimal resi-
dent stem/progenitor cells can contribute to endothelium for-
mation. These endothelial progenitor/stem-like populations
in the intima have potential to form neovessels and are able to
produce large numbers of endothelial cells both in vitro and in vivo. Because they are localized to the inner surface, they
can sense shear stress induced by blood flow. They may also
have a great potential for endothelial repair after damage asso-
ciated with turbulent shear stress in vivo.

As well as in the tunica media, populations of vascular
progenitors have also been found to be resident in the tunica
adventitia. Our group identified a Sca-1+ population of cells in the adventitia of the mouse aortic root (Figure 2). These cells formed ≤20% of the adventitial pop-
ation of the aortic root and were confirmed to differentiate to
smooth muscle cells in vivo. Clear evidence that these cells
can contribute to atherosclerotic lesions was seen in the fact
that when applied to the outer surface of a decellularized vein
graft these cells migrated through the wall, making up one
third of the cells of the neointima. Other groups have since
established similar cell populations that can be isolated from
the mouse mesenteric and femoral arteries. Zengin et al have
also identified a population of CD34+ cells in human arter-
ies that may play a similar role. Using a Wnt1-cre lineage
marker that identifies cells of neural crest origin, Passman
et al found that neither the adventitia nor Sca1+ cells were
labeled in arteries composed of neural crest–derived smooth
muscle cells. Development of the adventitia and maintenance
of a resident population of Sca1+ vascular progenitor cells in
the artery wall were correlated with activation of sonic hedge-
hog signaling. In adults, chimeric model of mouse bone mar-
row transplantation did not support the bone marrow origin of
vascular Sca-1+ progenitor cells. However, lineage-specific
tracking data are still lacking at present.
These examples show that many candidate cells exist that could be of great therapeutic benefit if we could harness their potential for vascular repair while suppressing their ability to contribute to harmful vascular remodeling. To exploit the benefits they offer, we must understand how these cells interact with the physical cues of their environment, such as the different biomechanical stimuli and the dynamic arterial and venous system.

Figure 2. Presence of vascular stem/progenitor cells in the vessel wall and circulating blood. Several types of stem/progenitor cells have been identified using a variety of markers. These cells are variously localized to the intima, media, and adventitia.

Biomechanical Forces in Development
It is well known that in normal mouse fetal development the heart begins to contract by 9.5 days; before the development of anything other than a primitive vasculature. It is thought that this contraction and its subsequent movement of hemangioblasts in the developing cell mass is the primary driver of differentiation of cells into the components of blood. Flow is also a driver in the remodeling of endothelial lined channels that are formed concurrently with the heart into the complex hierarchical network of the vascular tree. Lucitti et al showed that mature blood vessels do not develop unless there is viscous blood flow, and more recently, the same group has extended their study by showing the reasoning behind this phenomenon. High levels of blood flow from the heart toward the proximal arterial and venous system lead to fusion of early vessels to generate structures with a greater luminal area. In the absence of smooth muscle cells, which develop at a later stage of embryonic development, the endothelium has to be strong enough to withstand the effects of this flow and to do so must be able to form strong intracellular junctions. These junctions can be strengthened by the increasing volume of cells directly pushing on each other. This compressive force can affect local cell tension influencing cytoskeletal distribution. Thus, biomechanical force exerts its essential role in stem/progenitor differentiation into endothelial cells in the development of the vascular system.

Communication of Physical Forces

Flow Sensing Mechanisms
The precise mechanisms by which stem cells sense and translate shear stress into chemical signals leading to vascular lineage differentiation are not fully understood, but many models have been proposed. Donald Ingber’s model of cellular tensegrity suggests that the cell acts as a framework of cytoskeletal elements and anchoring integrins, which invoke a coordinated response to fluid flow communicated through a network of filaments. Disruption of this network leads to mechanical deformation of the nucleus, activating events leading to transcription factor recruitment. Integrins, when bound to their extracellular matrix substrates, act as mechanosensors to transduce physical force via recruitment of focal adhesion proteins that are connected to microfilaments, microtubules, and intermediate filaments and activate downstream signaling cascades. The glycocalyx has also been suggested as a sensory element, and it is proposed that patterns of fluid flow disrupting protruding glycoprotein strands may lead to disipation of shear stress, which is then communicated through the cell to initiate signaling cascades. Flow-responsive ion channels and G-protein–coupled receptors are additionally proposed means of shear responsiveness.

Signaling of Shear Stress–Induced Endothelial Differentiation
Evidence of biomechanical stress-induced stem cell differentiation has been widely reported. Shear stress is of particular interest in vascular cell remodeling because of its key role in endothelial activation and endothelial specification of stem cells. Several key signaling pathways have been proposed for stem/progenitor cell commitment to endothelial lineage. The most reported mechanism is the activation of vascular endothelial growth factor receptor-2 (VEGFR-2; also known as fetal liver kinase 1 [Flk-1] in mouse) by laminar shear stress in a ligand-independent manner. In several previous studies, the Flk-1 inhibitor, SU1498, has been reported to abolish shear stress-induced endothelial marker appearance in differentiating mouse ESCs. To further study the molecular mechanisms, it was reported that shear stress could activate Flk-1 in a ligand-independent manner. A handful of studies has reported that the PI3K–Akt pathway is an important master regulator in shear stress–induced endothelial differentiation of a variety of stem/progenitor cells, including human cord blood–derived progenitors, adipose-derived stem cells, and mouse ESCs. Interestingly, when floating-circulating phenotype endothelial progenitors derived from ex vivo expanded human cord blood were exposed to controlled levels of shear stress in a flow-loading device, the bioactivities of adhesion, migration, proliferation, tube formation, and differentiated type of endothelial cells increased. Several downstream effectors of PI3K–Akt have been suggested, of which...
histone deacetylases (HDACs) may play an important role. We and others have demonstrated that shear stress upregulates HDAC activity, and that HDAC inhibitors abolish flow-induced endothelial differentiation in adult progenitors and mouse ESCs.\(^{40,48}\) Rössig et al\(^{48}\) showed that this HDAC-mediated shear stress–induced endothelial differentiation may act by increasing expression and binding of transcription factor HoxA9 onto the endothelial nitric oxide synthase promoter. We have established HDAC regulation further by showing that laminar flow activates and stabilizes HDAC3, one of the class I HDACs, through the Flk-1–PI3K–Akt pathway and subsequently deacetylates p53 and activates p21 to induce mouse ESC-derived progenitor cell proliferation and differentiation into functional endothelial cells (Figure 2).\(^{40}\) Other factors such as sirtuin 1 (class III HDACs)\(^{49}\) and mammalian target of rapamycin\(^{50}\) have also been indicated as the downstream signal transducers of the PI3K–Akt pathway in the endothelial lineage commitment of human cord blood–derived progenitors. Recently, Dunn et al\(^{49}\) reported that flow can alter genome-wide methylation resulting in endothelial gene expression. Another school of thought is that shear stress induces the arterial endothelial cell commitment of stem/progenitor cells. Both VEGF downstream signaling pathways, PI3K–Akt and PKC–MAPKK–ERK, have been shown to be involved in the shear stress–induced upregulation of arterial endothelial marker ephrinB2 in stem/progenitor cells through the activation of Notch signaling\(^{47}\) and transcription factor Sp1.\(^{50}\) Additionally, an endothelial-selective transcription factor Hath6 was recently shown to be an important shear stress–responsive factor in shear stress–induced endothelial differentiation of human ESCs through targeting endothelial nitric oxide synthase expression.\(^{51}\)

Other mechanosensory mechanisms in flow-induced endothelial differentiation of stem/progenitor cells have also been suggested. Extracellular matrix–bound integrins are important mechanosensors that have been shown to mediate the laminar flow–induced endothelial commitment of rat bone marrow–derived endothelial colony-forming cells.\(^{52}\) The same research group has further suggested that cell cytoskeletal rearrangement, mediated by the activation of integrin β1, Ras, ERK1/2, paxillin, and focal adhesion kinase cascade, is essential in regulating this process (Figure 3).\(^{53}\) Because integrin expression is upregulated by VEGF during angiogenesis in mature endothelial cells,\(^{54,55}\) it is plausible to suggest that VEGFR-2/Flk-1 might act as a master switch that transduces signals through multiple downstream pathways to induce endothelial differentiation of stem/progenitor cells on encountering laminar shear stress. The glycocalyx component heparin sulfate proteoglycan has also been implicated as an alternative mechanosensor mediating shear stress–induced endothelial differentiation.\(^{56}\) Modeled microgravity has additionally been suggested as another type of mechanical stress that induces endothelial differentiation of rat bone marrow–derived mesenchymal stromal cells.\(^{57}\) We are starting to get a glimpse into the mechanisms underlying this phenomenon, but further efforts are required to clarify the full picture of intricate interactions between the various mechanosensory molecules in this biomechanical stress–induced stem/progenitor cell commitment to the endothelial lineage.

The downstream signal transducer for shear stress involves in transcription factor that mediates endothelial-specific gene expression. Recently, Fang et al\(^{58}\) reported the role of Hath6 in the endothelial specification of ESCs in response to shear stress. Hath6 (ATOH8), an endothelial-selective basic helix-loop-helix transcription factor, was first identified as a flow-responsive gene through a transcriptional profile analysis of human umbilical vein endothelial cells exposed to sustained laminar shear stress. In human ESC differentiation into endothelial cell models, Hath6 mRNA was upregulated synchronously with endothelial determination. Hath6 also facilitated the maturation of endothelial cells, tubular structure formation, and cell migration.\(^{59}\) Another well-known element in the promoter regions of endothelial-specific genes, for example, endothelial nitric oxide synthase and platelet endothelial cell adhesion molecule 1, is shear stress–responsive elements.\(^{59}\) Fluid shear stress regulates binding of mechanosensitive transcription factors, such as nuclear factor κB and early growth response 1, to shear stress–responsive elements in endothelial cells, where they maintain blood vessel homeostasis.\(^{59}\) These findings suggest that the transcriptional regulation that is necessary for pluripotency can be maintained and modified by the local micromechanical environment in stem cells.

**Signaling of Cyclic Stretch Induced Smooth Muscle Cell Differentiation**

As described above, resident stem/progenitor cells exist across the vessel wall. These cells play an important role in vascular homeostasis that is largely driven by biomechanical forces and injury insults. In pathological conditions such as angioplasty and vessel bypass surgery, they are also found to be contributory to vascular remodeling events.\(^{60}\) There is evidence that the cyclic strain generated by the pulsatile nature of flow throughout 1 cardiac cycle may be responsible for maintaining smooth muscle cells within the wall in an active, contractile status rather than in a proliferating one,\(^{61}\) and it may play a similar role in ensuring the quiescence of resident stem/progenitor cells. Strain within the vessel wall is heterogeneous. Circumferential strain is present where the vessel is straight, whereas at curvatures and...
branch point areas, known to be prone to the formation of atherosclerotic lesions, strain is more isotropic. It is possible that changes in strain may be responsible for the aberrant proliferation of smooth muscle cells associated with plaque formation. Related to this pathological phenomenon is the fact that mechanical stretch results in mesenchymal stromal cell differentiation into a smooth muscle phenotype. Such mechanical loading regulates smooth muscle marker gene expression, leading to differentiation into smooth muscle cells without the addition of growth factors. Using the different dominant mechanical stimuli, Maul et al found that cyclic strain in the vasculature alters stem cell morphology, growth, and differentiation. They demonstrated that mesenchymal stromal cells are sensitive to different types of forces and will respond in a dose-dependent manner to both the magnitude or frequency of cyclic strain and shear stress, but display a relatively constant phenotype on exposure to other forces such as cyclic hydrostatic pressure. In a 3-dimensional model, progenitor cells can differentiate toward a smooth muscle cell–like lineage when exposed to biomechanical stimulation. Others have shown that the absence of appropriate stretch is also linked to the development of osteogenic features in smooth muscle cells, showing that the level of tensional loading is important. Thus, it seems that cyclic strain stimulates stem/progenitor cell preferential differentiation into smooth muscle lineages.

Precise mechanisms of sensing in stem/progenitor cells and the complete pathways relating specific mechanical stimuli to stem cell fate remain to be elucidated. So far, no stem cell–specific stretch receptors have been identified. As discussed earlier, extracellular matrix–bound integrins are important in laminar flow–induced endothelial differentiation; however, they are also important in mediating cyclic strain–induced stem cell differentiation into smooth muscle cells. A second pathway initiated by mechanical stretch is platelet–derived growth factor receptor activation, which might initiate ESC differentiation into smooth muscle cells through the activation of Ras/ERK1/2 cascade. It was demonstrated that cyclic strain can directly stimulate platelet–derived growth factor receptor activation without ligand binding. Possibly, mechanical force might change platelet–derived growth factor receptor structure conformation leading to kinase domain exposure and consequently activate downstream signal transducers. Finally, it is known that both mechanical stimulation and transforming growth factor–β signaling play unique and important roles in the regulation of progenitor differentiation into smooth muscle cells at both transcriptional and post-transcriptional levels, and that a precise combination of microenvironmental cues might promote this process. Mechanical stretch–induced stem cell differentiation processes involve different signaling molecules and pathways and most likely depend on the activities of several mechanical sensors and signal transducers. Concerning transcription factor–mediated smooth muscle–specific gene expression, it is known that myocardin/serum response factor complex can directly bind to CArG element of promoter regions in smooth muscle–specific genes, which was partially confirmed in mechanical stress–initiated muscle differentiation. Thus, strain/stress can stimulate smooth muscle differentiation from stem cells, but detailed molecular mechanisms are largely unclear.

Clinical Relevance and Perspective

Bioengineered Vessel Constructs

Knowledge of the physical forces that regulate stem/progenitor cell differentiation will be beneficial in utilization of such cells in the clinic. For instance, to generate functional vessels suitable for grafting, it will be useful to culture cells on a scaffold constructed from materials that are likely to promote differentiation into vascular cells while discouraging the formation of bone-like materials. When vascular progenitors are applied to both the inner surface and the adventitial side of a decellularized vessel scaffold, biomechanical forces can be sensed by these cells during pressure-driven perfusion with culture medium. These cells were differentiated into both endothelial and smooth muscle cells, that is, lamellar flow shear–inducing endothelial differentiation and cyclic stretch–inducing smooth muscle phenotypes in tissue-engineered vessels. There is evidence that cells may retain epigenetic memory that may allow them to be more responsive and speed up the differentiation process when in situ. Culturing cells under exposure to biomechanical forces may also improve the ability of the engineered vessels to withstand pressures when transplanted into the body.

Restenosis After Stenting

Angioplasty, the insertion of a wire stent into an occluded artery to increase blood flow, is one of the most commonly performed surgeries worldwide. However, it is not a conclusive solution to the problem because restenosis of the vessel is not uncommon. Restenosis rates have improved dramatically because bare metal stents were replaced with drug-eluting stents in most angioplasty surgeries. Some of the most common drugs used, sirolimus (Rapamycin) that elicits an anti-proliferative effect through its action on mammalian target of rapamycin, and paclitaxel that acts via the antimigratory stabilization of cell microtubules, have decreased restenosis rates from 22% to 2%. However, because of the large number of such surgeries performed per year, this 2% still represents a significant problem. One of the key causes of pathogenesis is altered blood flow after stenting, because the lumen size of the vessel differs from the original artery. Recent work suggests that much of this restenosis could be down to the invasion of Sca-1+ vascular progenitors. Sirolimus was found to induce progenitor cell chemotaxis via activation of CXCR4 and drive smooth muscle cell differentiation through ERK and β-catenin, which could contribute to restenosis and vascular remodeling. Through increased understanding of the biochemical and biomechanical pathways that drive progenitor proliferation, migration, and differentiation, we could ensure drugs that promote progenitor differentiation toward endothelial lineages are applied in future generations of drug-eluting stents.

Stem/progenitor cells present a valuable tool for tissue bioengineering in the vasculature. However, their activation in the setting of arterial disease and their contribution to adverse vascular remodeling remain problematic. A greater understanding...
of how these cells can be manipulated by physical forces may allow us to develop preventative strategies for their adverse behavior and harness their full therapeutic potential in treating cardiovascular disease.

Sources of Funding
Vascular stem cell research in the Xu lab is supported by the British Heart Foundation and Oak Foundation.

Disclosures
None.

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Role of Biomechanical Forces in Stem Cell Vascular Lineage Differentiation
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Arterioscler Thromb Vasc Biol. published online July 10, 2014;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

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