Blood flow and stem cells in vascular disease

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Abstract

It is well known that the altered blood flow is related to vascular diseases, including atherosclerosis, restenosis, and arteriosclerosis, which preferentially located at areas with the disturbed blood flow, suggesting that altered biomechanical stress may exert their effect on the vascular disease. Recent evidence indicated the presence of abundant stem/progenitor cells in the vessel wall, in which laminar shear stress can stimulate these cells to differentiate towards endothelial lineage, while cyclic strain results in smooth muscle differentiation. In line with this, it was evidenced that altered biomechanical stress in stented vessels may lead to ‘wrong’ direction of vascular stem cell differentiation resulting in restenosis. However, the underlying mechanisms are not well understood. In this article, we will give an overview of the effect of the local flow pattern on stem/progenitor cell differentiation and the possible mechanism on how the blood flow influences stem cell behaviours in the development of vascular diseases.

Keywords: Shear stress † Stem cells † Vascular progenitors † Vascular tissue engineering † Restenosis

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1. Introduction

Blood flow passing through the vessels generates two main forces: shear stress and cyclic strain stretch. Shear stress is the mechanical force created when a blood flow acts on the surface of the endothelium. The blood flow in the straight part of the arterial wall results in a high shear stress unidirectional disturbed flow. On the other hand, cyclic strain stretch created by blood pressure exerts its effects in the whole vessel wall by stimulating endothelial and smooth muscle cells. In the vascular system, acute changes in the mechanical forces created by the blood flow can lead to immediate alterations in vessel diameter, which could be mediated by released vasoactive elements, e.g. nitric oxide. Longer lasting, chronically altered biomechanical stress created by hypertension or other pathological conditions may result in adaptive changes of the arterial wall, e.g. vascular remodelling. The arterial wall, thus, may undergo a disease process, e.g. arteriosclerosis, in which stem cells may be involved with their mobilization and differentiation.

Emerging data provide strong evidence of the existence of vascular stem/progenitor cell populations in a variety of tissues. Intriguingly, some of these vascular progenitor cells are capable of differentiation into endothelial and smooth muscle cells, thereby participating in angiogenesis and vascular remodelling. In 2004, Hu et al. demonstrated the presence of vascular stem/progenitor cells in the vessel wall, which subsequently confirmed and developed by other groups, which mainly focused their studies on the mobilization and specific localization of the cells and their functional roles in both physiological and pathological conditions of arteriosclerosis.

Arteriosclerosis is characterized by endothelial dysfunction/damage and smooth muscle cell accumulation in the intima with or without lipid deposition, resulting in the thickening and stiffness of the arterial wall. Endothelium turnover and the proliferation of smooth muscle cells are important events in the pathogenesis of vascular diseases. Traditionally, it is believed that during the development of arteriosclerosis, neighbouring cells, such as mature endothelial and smooth muscle cells from the vessel wall migrate to replace dead endothelial cells through replication and assumption of synthetic phenotypes. However, recent data strongly suggest that new sources of endothelial and smooth muscle cells differentiated from stem cells recruited from the resident vascular stem cell niche, the blood circulation, and other sites may also participate in the lesion development.

The present review aims to provide mechanistic insights into the impact of biomechanical stress generated by the blood flow on vascular stem cell behaviours in the development of arteriosclerosis. We will specifically focus on the nature, characterization, and mechanisms of differentiation of vascular stem cells in diseased vessels and address the controversial issues in the research field.

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### Table 1 Summary of published reports on progenitor cells found in the vessel wall

<table>
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<th>Publication</th>
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<td>EC, mural cells, and myocytes</td>
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<td>Pasquinielli et al.35</td>
<td>Human thoracic aorta</td>
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<td>EC</td>
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<tr>
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<td>Human aorta and mammary arteries</td>
<td>Atherosclerotic lesion/ adventitia</td>
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<tr>
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<td>Mouse embryonic/adult arteries</td>
<td>Media-adventitia</td>
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<td>Liu et al.97</td>
<td>Human blood and transplant atherosclerotic vessels</td>
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<td>EC</td>
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</tr>
<tr>
<td>Pasquinielli et al.35</td>
<td>Human arteries</td>
<td>Media-adventitia</td>
<td>Adipogenic, chondrogenic, and leiomyogenic</td>
<td>Oct-4, Stro-1, Sca-1, and Notch-1</td>
<td>Oct-4, Stro-1, Sca-1, and Notch-1 found in vasculogenic niche. Total vessel wall isolated showed an expression of stem (Stro-1, oct-1, and Oct-4) and MSC lineages (CD44, CD90, CD105, CD73, CD29, and CD166)</td>
</tr>
</tbody>
</table>

Continued
2. Stem cells in the vessel wall

Adult vessels are composed of rather quiescent cells that can be activated as a result of endothelial injury and other insults. Recently, it was demonstrated that the vessel wall contains stem cells that could incorporate at sites of injury and differentiate into endothelial cells or migrate across the vessel wall and subsequently differentiate into smooth muscle cells in the intima (Table 1). In the arterial media of the rats, a report demonstrated a presence of a 'side-population' of progenitor cells by using a method that exclude the fluorescent dye Hoechst 33342. These cells are CD34 and Sca-1+ which express the membrane transporter ABCG2. In the intima of the vessel wall, early study by Ingram et al. reported that a population of endothelial progenitor cells (EPCs) exists within the endothe- lium, which express a panel of the progenitor markers. These resident endothelial progenitors have an ability to form a colony in single cell culture and can proliferate rapidly in vitro and in vivo. Recent evidence additionally indicates the presence of endothelial progenitor/stem-like population in the intima where they have potential to form neo-vessels. This population is dormant in the steady state, but possesses colony-forming ability, and is able to produce large numbers of endothelial cells in vitro and in vivo. Taken together, these findings suggest that resident progenitor cells in the vessel wall can contribute to endothelial regeneration, which could be a potentially important source of 'resident' stem cells responsible for repairing of injured vessels.

Similarly, it has been reported that a population of cells in the human vein and artery displayed a positive staining for mesenchymal lineage markers. Working on human vena saphena leftover from cardiac bypass graft surgery, Campagnolo et al. have identified a new population of pericyte progenitor cells, which resides around adventitial vaso vasaun. In situ, these cells express CD34 and are negative for endothelial markers. Selection for CD34+/CD31− cells and culturing in the presence of the serum gave rise to highly proliferative population of CD34− cells that co-expressed pericyte/mesenchymal antigens (desmin, vimentin, NG2, PDGFβ, CD44, CD90, CD105, CD29, CD49α, CD49b, CD13, CD59, and CD73) together with the stem cell marker Sox2 and were negative for CD146, CD31, CD133, c-kit, and CD45. Vena saphena-derived progenitor cells showed clonogenic and multilineage differentiation capacities, but they were not able to differentiate into endothelial cells. Notwithstanding, they were able to support angiogenesis by integrating into endothelial networks on Matrigel and mouse ischaemic limb and cardiac tissues, and by releasing pro-angiogenic growth factors and microRNA-132. Accordingly, culture expanded vena saphena-derived progenitor cells improved post-ischaemic vascular repair, blood flow recovery, and cardiac function in mice with myocardial infarct. Autologous transplantation of these cells is currently explored for translation to the clinical setting in patients with cardiac ischaemia.

Working on human cadaveric tissues, as demonstrated by Pasquinielli et al. angiogenic mesenchymal stromal cells are also present in the thoracic aorta, which are CD34 and c-kit positive. These cells also expressed mesenchymal stromal cell markers CD44+, CD90−, and CD105− in the culture. Additionally, the cells can form capillary-like structures as well. Interestingly, a current report from Li’s laboratory identified a new type of stem cells in the blood vessel wall, named multipotent vascular stem cells, which differentiate into neural cells and mesenchymal stem cell-like cells that subsequently differentiate into smooth muscle cells. The group proposes that lesional proliferative or synthetic smooth muscle cells are derived from stem cells, and do not derived from the de-differentiation of the medial smooth muscle cells. In a mouse model of vascular injuries, they demonstrated that resident stem cells, but not mature smooth muscle cells, are responsible for smooth muscle accumulation to form neointimal lesions. The Tang et al. challenged the traditional dogma of the smooth muscle de-differentiation from the contractile to the proliferative/synthetic phenotype. However, this concept has not been accepted by a group of experts in smooth muscle research, who believe that medial mature smooth muscle cells can proliferate and be phenotypic switching in response...
to vascular injury.38 We believe that the debate will promote the re-
search to further elucidate the roles of stem cells in vascular remodel-
ning and address the fundamental issue: is vascular disease a stem cell
disease?39 (Figure 1)?

In summary, it seems that vascular stem cells described by different
groups exhibited a variety of phenotypes, for which it may be explained
by several reasons. First, the tissues and methods used by different la-
boratories are variable from foetal mouse aortas to human saphenous
veins. Secondly, a heterogeneous population of stem/progenitor cells
might exist in different layers of the vessel wall. Finally, there is no spe-
cific marker to identify ‘vascular stem cells’. In general, one population
described is multipotent stem cells expressing c-kit+, Sca-1+ and
Lin−11 or Sox17, Sox10, and S100B,37 which can differentiate into
many types of cells in vitro. The second well-described population is
mesenchymal stem cell characteristics including CD44+, CD90+, CD73+, CD34−, and CD45− cells.13 The third population is peri-
cytes expressing CD34, NG2, and PDGFRβ.30 In addition, there are
also other markers to identify stem/progenitor cells in the literatures.31
These results indicate the complexity of vascular stem cells, which could
be closely related to vascular repair and disease development.

3. Stem cells in blood

During last years, a large number of publications described adult vascu-
lar stem cells present in various kinds of tissues and organs.11,41 These
tissue resident stem cells might be released into circulation where they
form a stem cell pool, which has an ability to differentiate into vascular
lineage to repair damaged vessels or form new microvessels in damaged
issues.5 In this regard, bone marrow stem cells are intensively studied,
e.g EPCs.6 Classic ‘EPCs’ were firstly reported by Asahara et al.42 in
1997. Consequently, >2000 papers describing ‘EPCs’ were published,
which were recently identified to be monocytes/macrophages.43
However, several reports demonstrated the presence of ‘real’ EPCs
that have a nature of late growth in vitro and can integrate into the
vessel to form endothelial cells in vivo.44,45 Further study would be
needed to clarify the physiopathological role of bone marrow-derived
EPCs, if any. Additionally, Simper et al.46 provided the first evidence
that smooth muscle progenitors exist in the periphery blood by cultur-
ning human mononuclear cells in the presence of PDGF-BB. Libby’s
group has also reported the presence of blood smooth muscle progeni-
tors as identified by culturing blood CD14/CD105+ cells.47 Moreover,
Deb et al.48 showed that blood smooth muscle progenitors cells
express high levels of beta1-integrin when compared with endothelial
outgrowth cells. Moreover, proteomic analysis comparing the secre-
tome of blood smooth muscle progenitors and blood-derived late-outgrowth smooth muscle cells revealed that the progenitor cells
produced fewer proteolytic enzymes and inflammatory cytokines and
showed reduced invasive capacity.49 The progenitor cells secreted
extracellular matrix proteins as well as serum proteins, including pro-
teoglycans, and pigment epithelium-derived factor, a potent inhibitor
of angiogenesis in vitro. As a functional consequence, their conditioned
medium was less angiogenic in comparison with the secretome of
adult human aortic smooth muscle cells, as demonstrated by endothelial
tube formation assays in vitro and the implantation of Matrigel plugs into
immunodeficient mice.49 Cumulatively, the aforementioned results
support the novel concept that smooth muscle progenitors exist in
the human-circulating blood and may contribute to the pathogenesis
of vascular diseases.

4. Shear stress-induced stem cell
differentiation into endothelial
lineage

The lifespan of endothelial cells in the arterial wall is limited, e.g. ≏12
months in the areas with the laminar flow, but cells in the regions with
the disturbed blood flow may live only weeks.50 This indicates that dif-
ferent flow patterns could lead to a variety of endothelial turnover
rates.51,52 In support of this concept, recent study by Foteinos
et al.26 demonstrated that the numbers of death/proliferating cells
on the aortas of apoE−/− mice were significantly different to wild-
type animals. Importantly, lesion-prone areas display a higher turnover
rate of endothelial cells as indicated by BrdU positive staining. There-
fore, endothelial cells in these affected areas undergo a higher death/
proliferation cycle in the presence of hyperlipidaemia and disturbed
blood flow. We found that the disturbed flow can also result in endo-
thelial apoptosis, which is mediated by endoplasmic reticulum stress
response via the activation of X-box binding protein 1 (XBP1).53
We demonstrated that VE-cadherin is a down-stream target of
XBP1, i.e. XBP1 directly binding to the promoter of the gene. Therefore,
down-regulation of VE-cadherin may contribute to
XBP1-induced endothelial apoptosis induced by the disturbed blood
flow (for the review, see Xu54). Following endothelial death, it was
believed that adjacent endothelial cells proliferate and migrate to
replace the dead cells.55 However, recent evidence indicates the
impact of vascular stem/progenitor cells in endothelial regeneration
in the areas with endothelial denudation.56 As described above,
recent studies indicated that multipotent vascular stem cells exist in
the blood and vascular wall and contribute to tissue regeneration in the responses to vascular injury (for review, see Pacilli and Pasquinielli). How can these stem cells become endothelial cells to repair damaged vessels? At the present, it is believed that shear stress is crucial for the initiation of stem cell differentiation towards endothelial lineage.

4.1 Effect of shear stress on endothelial differentiation

The local microenvironment determines the cell fate and cellular behaviour. Endothelial cell differentiation of vascular progenitor cells is, therefore, influenced by local flow pattern and shear stress. Several groups have provided strong evidence that pulsatile/non-pulsatile laminar shear stress (6–15 dynes/cm²) enhances the endothelial differentiation of stem/progenitor cells, including embryonic stem cell, 6,61 embryonic stem cell-derived Sca1⁺ or Flk-1⁺ cells, 62,63 mesenchymal stem cell, 64,65 mesenchymal progenitor cells, 66,67 EPCs 68–70 isolated from bone marrow, peripheral blood, or cord blood, and multipotent stem cells isolated from the adipose tissue 71 or the placenta. 72 Compared with cells under static conditions, the sheared cells exhibited much higher endothelial-like phenotype, including the elevated expression of endothelial marker proteins, such as CD31, CD144, eNOS, and vWF, etc., increased capacity to form capillary-like structures in vitro and neo-vessels in Matrigel implantation in vivo. Most importantly, the differentiated endothelial cells could incorporate into the injured femoral artery, repair the injured endothelium and reduce injury-induced neointima formation in ApoE⁻/⁻ mice. 62 Meanwhile, it was found that the laminar flow suppressed stem/progenitor cell differentiation towards smooth muscle lineage, evidenced by significantly decreased expression of a smooth muscle marker, such as smooth muscle alpha-actin, calponin, and SM22. However, two studies reported that both endothelial and smooth muscle cells can be derived by embryonic stem cells submitted to shear stress. For example, Illi et al. 69 demonstrated that 10 dynes/cm² shear stress is enough to induce endothelial and smooth muscle marker expression. Similarly, Huang et al. 59 reported that a combination of a wall shear stress from ~0.98 to 2.2 dyn/cm² and a circumferential strain stress 4.6–9.6 × 10⁴ dyn/cm² in a compliant microporous polyurethane tube results in stem cell differentiation into endothelial and smooth muscle cells. In such tubes, the differentiated cells grew in 3D dimensions along the tube surface and into the interstices. After flow treatment, the inner layer of the cells displayed endothelial-like appearance, and the deeper layer of the cells is stained positive for smooth muscle markers. These results indicate the potential of stem/progenitor cells to differentiate into both endothelial and smooth muscle cells at the same time through modulating the differentiation procedures. Different observations among different groups may be due to the methodological variations.

Flk-1 and/or Sca-1 are progenitor markers, usually used to isolate vascular progenitors from murine stem cell populations undergoing spontaneous differentiation in the absence of LIF and the presence of VEGF. A study by Zeng and colleagues 65 demonstrated that Sca-1⁺ cells could differentiate into endothelial lineage under VEGF treatment or laminar flow with higher efficiency when compared with unselected differentiating stem cells, while the smooth muscle lineage differentiation was suppressed by the flow. A similar result was reported on isolated Flk1⁺ cells by Yamamoto et al. 63 When compared with the static control, the expression of endothelial markers (Flk1, Flt1, VE-cadherin, and PECAM-1) and the accumulation of cells in the S and G2/M cell cycle phases were significantly increased in Flk1⁺ cells under the laminar flow, thus suggesting that the laminar flow enhances Flk1⁺ cell proliferation and differentiation towards endothelial lineage. The sheared progenitor cells showed an endothelial-like appearance with orientation along the flow direction, and could form capillary-like structures on Matrigel surface. 74 Whatever sources derived from, vascular progenitor cells are, at least, bipotent, able to differentiate into either endothelial or smooth muscle lineages depending on the differentiation procedures. 77,78 Taken together, the results indicate that laminar shear stress results in stem/progenitor cell differentiation towards endothelial phenotypes, while static situation leads stem cells to smooth muscle differentiation (Figure 2).

4.2 The mechanism of shear stress-induced endothelial differentiation

Cell differentiation is the result of multi-factors and multi-step process, the underlying mechanism of which is still unclear. Similar to mature endothelial cells, the VEGF receptor Flk1 in stem cell-derived progenitor cells can be activated by the laminar flow in a...
ligand-independent manner, which is essential for flow-induced endothe-

telial differentiation. In a recent study, the Flk1 inhibitor, SU1498, 

ablaited laminar flow-induced endothelial marker appearance in differ-

entiating stem cells. Similar results were observed by Yamamoto et al., who reported that SU1498 blocked shear stress-induced Flk1+ progenitor differentiation towards endothelial cells, while a 

neutralizing antibody against VEGF did not. These results suggest that a ligand-independent activation of Flk1 is essential for endothelial differentiation under flow. Several down-stream molecular signalling can be triggered by Flk1 activation, one of which is the PI3K/Akt pathway. PI3K/Akt activation was reported essential for flow-induced stem cell differentiation towards endothelial lineages. Similarly, Ye et al. showed that laminar shear stress-induced differentiation of human cord blood progenitors towards endothelial lineage was also dependent on Akt activation. As to PI3K/Akt down-stream effectors, histone deacetylases (HDACs) may play an important role. The first indirect evidence for HDACs involvement in vascular growth derived from using the Trichostatin A (TSA) HDAC inhibitor, which blocked hypoxia-induced angiogenesis in tumour tissue. There is evidence that the laminar flow up-regulated HDAC activity in stem cells and endothelial progenitors, involved, as siRNAs for HDAC1 and HDAC3 inhibit endothelial differentiation induced by the laminar flow. It seems that the down-stream targets of HDACs involving in PI3K-Akt and in-

directly activation of p53/p21, which may increase differentiated endo-

thelial survival, therefore, contributing to stem cell differentiation.

5. Strain stress-induced stem cell differentiation into smooth muscle cells

Blood vessels are constantly exposed to cyclic stretch induced by pul-

satile luminal pressure. In pathological conditions such as acute 

hypertension and during angioplasty, stem cells of the arterial wall 

are stretched. Evidence accumulated during the past years contribu-

ted to demonstrate that mechanical stimuli regulate stem cell differen-

tiation and function as well as vascular remodelling. Research has 

been performed on effects of cyclic stretch on stem cell differenti-

ation, which are exposed to such forces in vitro. With cyclic strain, stem cells displayed a spindle-shaped morphology and parallel 

alignment. Cells exposed to cyclic strain showed significantly 

increased gene and protein levels of smooth muscle cell markers. 

Additionally, cyclic stretch led to the differentiation of bone 

marrow stem cells to differentiate into smooth muscle-like cells 

without the addition of the growth factor. Similarly, Flk-1+ progeni-

tor cells seeded on flexible silicone membranes were subjected to 

controlled levels of cyclic strain, which significantly increased the 

expression of smooth muscle markers, e.g. smooth muscle alpha-actin 

and smooth muscle-myosin heavy chain, whereas the expression of the 

vascular endothelial cell marker Flk-1 decreased. The PDGF 

receptor-beta kinase inhibitor AG-1296 completely blocked the 

cyclic strain-induced increase in cell number and smooth muscle 

marker expression. Cyclic strain immediately caused phosphorylation 

of PDGF receptor-beta in a dose-dependent manner, but neutralizing 

antibody against PDGF-BB did not block the PDGF receptor-beta 

phosphorylation. These results suggest that cyclic strain activates 
PDTG receptor-beta in a ligand-independent manner and that the 

activation plays a critical role in smooth muscle cell differentiation 

from stem cells.

6. Biomechanical stress is crucial for creation of tissue-engineered 

vessels ex vivo

As described above, shear stress results in stem cell differentiation 

towards endothelial lineages, while cyclic strain leads to smooth 

muscle phenotypes. According to this model, it is reasonable that a 
tissue-engineered vessel could be created in a scaffold by seeding 

stem cells and using different mechanical forces. Vascular tissue engi-

eering research involves scaffold preparation, cell application in vitro 

and application to an animal model for in vivo studies. It has been 

shown that stem cells have the potential to differentiate into endothe-

lia and smooth muscle cells in response to biomechanical stress, thus 

providing a potential cell source for vascular tissue engineering. Mar-

gariti et al. recently established a method to generate partially 

induced pluripotent stem cells by direct reprogramming fibroblasts 

to vascular cells. A decellularized vessel was used as the scaffold ma-

terial to create a tissue-engineered vessel with a flow system to gen-

erate shear stress and strain stretch ex vivo. In this system, stem cells 

were seeded on the adventitial side of the decellularized vessel scas-

fold in a specially constructed bioreactor in medium. After applying 

strain stress with the flow system, stem cells were seeded in the 

lumen side of the scaffold, to which shear stress was applied. At 1 

week after cell seeding, the bioengineered vessel was harvested and 

subjected to in vitro analyses. It stained positive for a number of 

smooth muscle markers and exhibited the characteristic morphology 

and the localization of smooth muscle cells in the media of the vessels. 

Endothelial positive staining was observed in the lumen side of the 

tissue-engineered vessels. This result indicates that ex vivo biomech-

anical stress leads to vascular lineage differentiation of stem cells, 

which is useful for the generation of tissue-engineered vessels (Figure 3).

7. Altered biomechanical stress-related restenosis in stented 

vessels

Percutaneous coronary interventions, including stent insertion, are 
established therapies in both acute coronary syndromes and symp-
tomatic chronic disease refractory to pharmacological therapy. These continually advancing treatments remain limited by restenosis or thrombosis of vessel segments in stented vessels caused by neo-
timal hyperplasia, impaired endothelialisation, and accelerated athero-
sclerosis. Neointimal growth is a multifactorial response to mechanical 

vessel injury at the time of angioplasty and stenting. The 

radial force required to dilate the delivery balloon and deploy a 

stent results in fracturing or tearing of atherosclerotic plaque, endo-

thelium, and the intimal and medial layers of the artery wall. This 

results in a cascade of cytokine and growth factor release, expression 

of adhesion molecules, recruitment and infiltration of macrophages 

and other inflammatory cells, smooth muscle proliferation and migra-

tion, and matrix protein deposition. Neointimal development leads to 

luminal narrowing, reduction in the coronary blood flow, thus
Importantly, the administration of a granulocyte-colony stimulating factor to patients after coronary stenting led to elevated levels of blood stem cells as well as increased restenosis.  However, there is no direct data able to clarify the underlying mechanisms. Nonetheless, we postulate that shear stress is low in the stented vessel where stem cells migrated to the surface of damaged areas after stenting. These stem cells may not sense the shear stress and, consequently, they may not differentiate into endothelial cells properly. In turn, stem cells may differentiate into smooth muscle cells and form neointimal lesions. Thus, altered blood flow and biomechanical stress locally in the stented vessels could influence the fate of resident stem cell differentiation, which is unfavourable to heal the damaged endothelium, rather resulting in smooth muscle cell accumulation.

8. Summary and perspectives

The present review has briefly summarized the recent results on how blood flow influences stem/progenitor cell differentiation into endothelial and smooth muscle cells, which is a key event in the development of vascular diseases. The laminar flow can stimulate stem/progenitor cells to differentiate into endothelial phenotypes involving the signal transduction pathways of VEGFR-PI3K-Akt-HDACs, while strain stretch enhances smooth muscle differentiation through the down-regulation HDAC7/8 and TGF-β pathway. Based on our knowledge of different types of stem cells that can be primed to differentiate towards vascular cells in response to laminar flow and stretch stress, we could design a bioreactor system to create a tissue-engineered vessel. Altered haemodynamic flow and variations of shear stress in the stented vessels involved in restenosis might be responsible for stem cell behaviours where they differentiate into smooth muscle cells. Current knowledge has been accumulated by research on stem/progenitor cell differentiation useful for cell-based therapy for vascular regenerative medicine. However, there are several questions concerning how the blood flow influences stem cell functions in physiology and pathological conditions, which remain to elucidate. For example, whether the disturbed flow areas serve as stem cell reservoir where they constitutively proliferate. As identified in a recent study, proliferating cells in disturbed flow areas may actually be stem/progenitor cells in response to local or distal injury stimuli. If it is true, we can postulate that vascular disease is, at least, in part, a stem cell-related disease. On the other hand, stem cells in circulation and the vessel wall can differentiate into both endothelial and smooth muscle cells and also stimulated by both shear and stretch stresses in vivo. How do the stem cells sense the mechanical stimuli to go to the specific direction? In another word, how do they know which signal to obey in vivo? At the present, there is no direct evidence to answer the question. Further investigation on this issue would enhance our understanding of the mechanism of stem cell differentiation providing some basic information for the application. In addition, accumulating evidence indicates abundant stem/progenitor cells in a variety of vessels, including artery, vein, and microvessels. Can stem/progenitor cells resident in the vessel wall be released into the circulating blood to form the stem cell pool in response to altered biomechanical stress? Finally, it is unknown if pathophysiological process involving altered blood flow could trigger stem cell mobilization and differentiation in a ‘wrong’ direction. The answers to these questions will enhance our knowledge on vascular biology and the mechanisms involved in blood flow-related stem cell functions in vascular diseases.

Figure 3: A flow system serves as a bioreactor for vascular tissue engineering. Vascular progenitor cells can be seeded on decellularized vessel scaffold, which is placed in a flow culture system. Shear stress is applied from low to high (0–15 dyes/cm²) within 48 h. The pressure is maintained at 150 mmHg for a week. In response to shear stress, vascular progenitors located at inner surface differentiate into endothelial cells, whereas the cells in the media sensing to stretch stress can differentiate into smooth muscle cells. Thus, a tissue-engineered vessel can be created based on the mechanisms of stem cell responding to biomechanical stress.
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