GENERATING NEW BLOOD FLOW:
INTEGRATING DEVELOPMENTAL BIOLOGY
AND TISSUE ENGINEERING

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ABSTRACT

Vascular tissue engineering aims to restore blood flow by seeding artificial tubular scaffolds with endothelial- and smooth muscle cells, thus creating bioartificial blood vessels. Herein, the progenitors of smooth muscle and endothelial cells hold great promise because they efficiently differentiate and harbor longevity. In this review we describe a novel tissue engineering approach which utilizes current insights from developmental biology, i.e. progenitor cell plasticity, and the latest advances in biomaterial design. We focus specifically of developmental processes that regulate progenitor cell (trans)differentiation and offer a platform for the integration of these molecular clues into biomaterial design. We propose a novel engineering paradigm for the creation of a small-diameter blood vessel wherein progenitor cell differentiation and tissue organization are instructed by the biomaterial solely. With this review we emphasize the power of integrating developmental biology and material science for vascular tissue engineering.

INTRODUCTION

Regenerative medicine is a burgeoning field that aims to improve health by restoring, regenerating or repairing diseased, damaged, or malfunctioning tissues. In vascular tissue engineering, blood flow is regenerated by bioartificial replacement vessels that are engineered to replace damaged vessels, e.g. in peripheral vascular disease and coronary heart disease. Ideally, the replacement vessel resembles its healthy native counterpart. In traditional vascular tissue engineering, replacement vessels are engineered in vitro by seeding (polymeric) tubular scaffolds with autologous endothelial cells (EC) and smooth muscle cells (SMC) to produce an anti-thrombogenic conduit that is not immunogenic. In principle, an in vitro tissue engineered blood vessel (TEBV) resembles a native small-diameter (≤ 6 mm internal diameter) arteries and must be able to withstand physiological pressure changes. Hence, the development of a viable TEBV depends on a suitable biomaterial that provides the appropriate architecture and mechanical strength, and augments cellular attachment and the seeding of SMC and EC. The concept of a living, functional TEBV requires the TEBV to respond to its environment, to be remodeled by the host and, most important, self-repair when damaged.

Stem cells and progenitor cells hold great promise for vascular tissue engineering, as they hold the key to tissue development and longevity, and thus self-maintenance. Endothelial progenitor cells (EPC) can be isolated from the peripheral blood and differentiated in vitro into mature and functional vascular cells (reviewed in [190]). Furthermore, EPC display great plasticity, meaning that they can differentiate into functional endothelial cells, but also transdifferentiate into contractile smooth muscle cells, depending on the molecular clues provided.

During embryogenesis, especially during the development of the heart [191] and pulmonary and aortic arteries [192;193], SMC are derived from EC through a process termed endothelial-to-mesenchymal transdifferentiation (EnMT). In extrapolation, this implies that both EC and SMC can be derived from a single progenitor cell pool, the EPC, and may thus reduce laborious isolation and culturing periods that hamper current vascular tissue engineering approaches. The application of EnMT in vascular tissue engineering may therefore lead to a more powerful tissue engineering strategy. However, before we introduce such developmental processes to vascular tissue engineering, it is essential to decipher the control mechanisms that regulate EnMT.

Therefore, the first part of this review outlines the current understandings of EnMT by EC and their progenitors. It focuses on the signaling events involved in EnMT and the cellular processes they evoke. The second part discusses current developments in ‘smart biomaterial’ design, providing perspectives on the incorporation of EnMT in future vascular tissue engineering approaches.

ENDOTHELIAL PROGENITOR CELLS

Circulating EPC were first described a mere decade ago as a subset of the CD34+ hematopoietic progenitor cells. In their landmark paper, Asahara et al. showed that bone marrow-derived mononuclear cells, enriched for either CD34 or vascular
endothelial growth factor receptor-2 (VEGFR-2), could differentiate into endothelial-like cells when cultured on fibronectin and in the presence of angiogenic growth factors [46]. Ever since, EPC have been at the center of vascular research and their potential in regenerative medicine is currently undoubted and well anticipated [194].

In the decade following the discovery of the archetype CD34+ EPC, a second type of EPC has been described, which can be distinguished through clonal and functional analysis [63]. The ‘early outgrowth EPC’, also known as ‘colony-forming unit endothelial cell’ [74], ‘colony-forming unit Hill’ [195] and ‘endothelial-like cell’ [101], are derived from the monocytic (CD14+) cell lineage and co-express endothelial cell markers, such as CD31, CD105, CD144, vWF, eNOS and Tie-2, and markers of the myeloid lineage, such as CD14, CD14 and CD45 (Figure 1). In culture, early outgrowth EPC form colonies from which spindle-shaped cells originate. Early outgrowth EPC display transient proliferative potential [101;113] and overlap between endothelial cell and monocyte/macrophage cell functions, i.e. phagocytosis [196], antithrombogenic activity [113;197], and the production of vasoactive mediators [101;113].

The archetype ‘late outgrowth EPC’, also known as ‘endothelial colony forming cells’ [47], is thought to originate from the CD34+ hematopoietic stem cell and exclusively expresses markers of the endothelial cell lineage after cell culture. Late outgrowth EPC form colonies of cobblestone-appearence that morphologically resemble microvascular endothelial cells and display an almost indefinite proliferation potential [198], indicative of their self-renewal capacity. This self-renewal is of major importance when engineering a viable and self-sustaining TEBV.

Despite the obvious in vitro distinction between ‘early outgrowth’ and ‘late outgrowth’ EPC, there is abundant evidence that both types of EPC can replace damaged endothelium in vivo and contribute to neovascularization in animal models of vascular damage, although there is debate on the precise mechanism of action [61;199]. The relatively high numbers of ‘early outgrowth’ EPC in the circulation may indicate that these cells primarily respond to acute damage of the endothelium [87], whereas the highly proliferative nature of ‘late outgrowth’ EPC may suggest a role in neovascularization [200;201] where a large number of cells is needed to form the neovessels. Moreover, recent data suggest that ‘early outgrowth’ and ‘late outgrowth’ EPC cooperate through paracrine signaling and act synergistically to stimulate neovascularization and endothelial cell formation [113;202].

For the purpose of this review, we do not discriminate between the origins of EPC-derived endothelial cells. Here, we focus on EPC and their progeny EC, which minimally comply to the following criteria; expression of endothelial cell markers (i.e. CD31, CD144, eNOS, vWF and Tie-2), proven antithrombogenic behavior, and the ability to form neovessels in vivo.

ENDOTHELIAL-TO-MESENCHYMAL TRANSDIFFERENTIATION (ENMT)

Traditionally, EC and SMC were thought to originate from spatially and temporally distinct cell sources during (embryonic) blood vessel development. However, accumulating experimental evidence indicates that EC contribute to the formation of SMC through a process termed endothelial-to-mesenchymal transdifferentiation (EnMT). During embryonic development, for instance the development of coronary vessels and heart valves, SMC are derived from the epicardial cells that undergo EnMT [192;193].

In addition to EnMT observed during embryonic development, the capacity of EC to transdifferentiate into SMC was shown in cardiac fibrosis [166], arteriosclerotic neointimas [203] and intimal thickenings found in lesions formed by pulmonary hypertension [204;205]. Overall, the notion that cells from the endothelial lineage can differentiate into SMC provides an interesting perspective for vascular tissue engineering (described below) and challenges the current dogma that vascular endothelial cells are terminally differentiated.

ENMT STARTS WITH INCREASED TGF-ß SIGNALING AND LOSS OF ENDOTHELIAL CELL-CELL CONTACTS.

EnMT is a multifactorial process in which several membrane-bound receptors and signal transduction pathways converge (Table 1). However, the transforming growth factor-ß (TGF-ß) superfamily is pivotal in EnMT.
Tissue injury, inflammation and hypoxia all lead to increased production of TGF-β by cells surrounding the endothelium. Increased TGF-β signaling reduces the integrity of the endothelial cell monolayer, either by the induction of endothelial cell apoptosis or by the depression of endothelial cell-cell contacts (Figure 2A). In this respect, TGF-β was shown to induce apoptosis specifically in EC through cross-talk between the VEGF165/VEGFR-2-induced activation of p38/MAPK and TGF-β signaling cascades [153;154](Figure 2B).

Furthermore, recent studies provide evidence that loss of endothelial adhesion molecules consistently preceded changes in endothelial morphology consistent with EnMT, which may result from combined signaling through TGF-β and Notch [145] (Figure 2B). However, the functional role of TGF-β in the regulation of EC adhesions has yet to be fully elucidated.

Taken together, EC apoptosis and the disruption of endothelial cell-cell contacts result in the selection of cells which are responsive to TGF-β signaling and primed for EnMT.

### Table 1. Initiators, modulators and inhibitors of EnMT.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>EnMT Initiating action</th>
<th>EnMT Inhibitory action</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Growth Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMP-7</td>
<td>↓ TGF-β, ↓ ECM production</td>
<td></td>
<td>[166,369]</td>
</tr>
<tr>
<td>EGF</td>
<td>↑ mesenchymal cell proliferation</td>
<td></td>
<td>[369]</td>
</tr>
<tr>
<td>aFGF</td>
<td>↓ actin filaments, ↓ Activin A,</td>
<td></td>
<td>[155]</td>
</tr>
<tr>
<td>BFGF</td>
<td>↑ TGF-β signaling, ↓ actin filaments</td>
<td></td>
<td>[144,370]</td>
</tr>
<tr>
<td>IGF-1</td>
<td>↑ TGF-β, signaling, ↑ actin filaments</td>
<td></td>
<td>[163]</td>
</tr>
<tr>
<td>IGF-2</td>
<td>↑ cell-cell contacts</td>
<td></td>
<td>[377]</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>↑ motility, ↓ actin filaments</td>
<td></td>
<td>[234,372]</td>
</tr>
<tr>
<td>TGF-β</td>
<td>↓ NO production, ↓ EC proliferation, ↓ cell-cell contacts, ↑ EC apoptosis, ↓ actin filaments, ↓ ECM production</td>
<td></td>
<td>[163]</td>
</tr>
<tr>
<td>VEGF</td>
<td>↓ TGF-β signaling, ↓ VEGF-α, ↑ ECM production</td>
<td></td>
<td>[205]</td>
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<td><strong>Adhesion molecules</strong></td>
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<tr>
<td>β-Catenin</td>
<td>↑ actin filaments</td>
<td></td>
<td>[191]</td>
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<tr>
<td>Endoglin</td>
<td>↑ AKT signaling, ↑ TGF-β, signaling, ↑ activin</td>
<td></td>
<td>[143]</td>
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<tr>
<td>VE-Cadherin</td>
<td>↓ motility, ↓ cell-cell contacts</td>
<td></td>
<td>[215-217]</td>
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<td><strong>ECM Components</strong></td>
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<tr>
<td>Hyaluronic acid</td>
<td>↑ motility</td>
<td></td>
<td>[369]</td>
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<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td>↓ NO, ↓ TGF-β, ↓ ECM production</td>
<td></td>
<td>[203]</td>
</tr>
<tr>
<td>Notch</td>
<td>↓ Delta, ↓ TGF-β, ↓ cell-cell contacts, ↓ actin filaments</td>
<td></td>
<td>[369]</td>
</tr>
<tr>
<td>Jagged-1</td>
<td>↓ cell-cell contacts, ↓ actin filaments</td>
<td></td>
<td>[145]</td>
</tr>
<tr>
<td>MMP-2 and MMP-9</td>
<td>↓ TGF-β signaling, ↓ VEGF-α, ↓ ECM production</td>
<td></td>
<td>[163,166,373]</td>
</tr>
</tbody>
</table>

EC = endothelial cell, BMP = Bone Morphogenic Protein, ECM = Extracellular Matrix, EGF = Epidermal Growth Factor, FGF = Fibroblast Growth Factor, IGF = Insulin-like Growth Factor, PDGF-BB = Platelet-derived Growth Factor, TGF = Transforming Growth Factor, NO = Nitric Oxide, VEGF = Vascular Endothelial Growth Factor, eNOS = endothelial cell NOS, MMPs = Metalloproteinases.

### CELL-MATRIX INTERACTION, ENDOTHELIAL CELL MIGRATION AND ENMT.

The acquisition of a migratory phenotype, preceded by changes in cell-matrix interaction, is the second phase in EnMT (Figure 2C). Already in 1987, Krug and coworkers recognized an important role for the extracellular matrix (ECM) as inducer of ‘cytodifferentiation’. In their report, extracts of ECM were produced by EDTA extraction and added to embryonic EC cultures. These stimulated EC displayed morphological changes and increased migratory activity consistent with EnMT [205]. Although no soluble factors were identified by Krug and coworkers, it is now well established that most ECM compounds participate in the regulation of cell adhesion, migration, growth and differentiation by storing and releasing growth factors and cytokines [207].

Cell migration depends on the degradation of the ECM by matrix metalloproteinases (MMPs), and although numerous studies have shown the involvement of MMPs in morphogenesis, cell migration and tissue remodeling, the role of MMPs in EnMT remains undefined. TGF-β induces production, secretion and activation of MMPs, especially MMP-2 and MMP-9 (Figure 2D). These MMPs degrade the ECM with some substrate specificity, allowing the partial degradation of the endothelial basement membrane [208;209], reducing cell-matrix interactions, and thus allowing cell migration (Figure 2C). Interestingly, recent results from Ishihara et al. indicate involvement of TGF-β and MMP-2 in the impairment of the endothelial cell barrier by reducing endothelial cell-cell contacts, thereby further increasing the motility of EC (Figure 2D).

Of further interest, TGF-β can modulate the expression and function of the main cellular receptors of ECM, i.e. the integrins. Binding of an integrin to its ECM ligand causes focal clustering of the integrins within the cell membrane and initiates many signaling pathways (reviewed in [211]). Interestingly, the role for TGF-β in the regulation of integrin expression is twofold. One the one hand, TGF-β reduces the expression of integrins that bind the endothelial basement membrane, i.e. α1β1 and α2β1 (Figure 2D), through the disruption of focal adhesion kinase/Src complexes [212;213]. On the other hand, TGF-β increases the expression of integrins that bind collagen type I, e.g. α1β1, (Figure 2D). Together, this changes the ECM binding specificity of cells in EnMT from an ECM preferred by EC to a matrix favored by SMC.

In summary, TGF-β signaling increases cell motility through disruption of endothelial cell-cell adhesions and the destruction of the endothelial basement membrane. Therefore, TGF-β stimulates cell migration by a shift in integrin expression changing the matrix preference of cells in EnMT from a preference for the endothelial basement membrane to the collagen(s) preferred by SMC.

### REGRESSION OF ENDOTHELIAL CELL MARKERS AND INCREASE OF SMOOTH MUSCLE CELL MARKERS.

The most palatable phenomenon in EnMT is the shift in cell marker expression. Although EnMT in general is driven by TGF-β, it is only partially involved during the phenotypic shift. The loss of endothelial marker expression is a downstream effect of the events initiated by TGF-β (Figure 2F). TGF-β induces signaling cascades which effectively prevent the production of EC-associated proteins. Subsequently, the resident EC marker molecules wane as a result of protein turnover.
Vascular endothelial growth factor (VEGF) is the most potent regulator of the endothelial phenotype. Signaling through its specific receptors induces expression of most EC-associated proteins and enzymes (reviewed in [214]). VE-cadherin clusters with the VEGF receptors on the cell membrane, where it stabilizes the availability of the VEGF receptors and modulates their downstream signaling [215,216]. In this way, VE-cadherin influences the expression of von Willebrand factor (vWF), CD31, endothelial cell nitric oxide synthase (eNOS), VEGFR-2 and itself [217].

Figure 2. Overview of the endothelial-to-mesenchymal transdifferentiation process. Schematic representation (A,C,E,G) and molecular mechanisms (B,D,F,H) behind the different phases in EnMT. (A,B) EnMT is initiated by increased TGF-β signaling in endothelial cells and loss of endothelial cell-cell contacts. (C,D) Endothelial cells in EnMT adopt a migratory phenotype and change their extracellular matrix preference. (E,F) Loss of endothelial cell marker expression and synthesis of the smooth muscle contractile apparatus. (G,H) Functionalization of the smooth muscle phenotype and alignment of synthetic smooth muscle cells.

Of note, integrins, the main receptors for extracellular matrix molecules, also form heterotypic complexes with several growth factor receptors. Endothelial-specific integrins α5β1 and αvβ3 were shown to associate with VEGFR-3, which in concert specifically increase endothelial-resistance to apoptotic stimuli [218] and the expression of eNOS [219], and thus directly affect EC function (Figure 2F).

In contrast to TGF-β’s indirect role in reducing the endothelial phenotype, TGF-β directly induces the expression of smooth muscle-specific genes. TGF-β signaling through its receptors TGF-βRI, TGF-βRII and TGF-βRIII, activates the Smad2/3 signaling cascade and induces the expression level of smooth muscle-specific genes [220,221]. Of particular interest, regions that account for tissue-specific transcription have been identified within several promoters of SMC-specific genes, including α-smooth muscle actin (αSMA) [222], smooth muscle protein 22α (SM22α) [223] and smooth muscle myosin heavy chain (SMMHC) [224]. These promoter regions can be activated by transcription factors such as GATA-5, GATA-6, MEF-2 and SRF [225], resulting in protein expression of the smooth muscle contractile apparatus (Figure 2F). Notably, all these transcription factors are associated to TGF-β/Smad signaling [226,227].

Taken together, TGF-β diminishes the expression of EC-specific molecules, not through direct interference, but by disrupting the signaling complexes that induce their expression, while TGF-β specifically induces the expression of the contractile apparatus of SMC. Hence, a shift in phenotype becomes prominent.

DIVERSIFICATION OF THE SMOOTH MUSCLE PHENOTYPE.

SMC can perform both contractile and synthetic functions. These contractile- and synthetic SMC differ in morphology, proliferation and migration rates and the expression of SMC marker proteins. Contractile SMC have low proliferation and low protein production rates, whereas synthetic SMC have reduced expression of the contractile apparatus proteins, but increased proliferation rates and increased extracellular matrix (ECM) production. The existence of SMC plasticity does not result from differences in origin or cell fate, but is a mere consequence of differential signaling events, allowing SMC to shift from the one cell state into the other [228,229] (Figure 2G).

Although TGF-β is the driving force behind SMC differentiation in general, and the production of the contractile apparatus in particular (Figure 2H), it is the platelet-derived growth factors (PDGFs) that are the main determinants of the synthetic smooth muscle phenotype (Figure 2H). PDGFs directly reduce protein expression of the contractile apparatus [230,231], and specifically increase protein expression of ECM proteins, e.g. collagens and fibronectin [232], functionally changing the smooth muscle phenotype from contractile to synthetic (Figure 2H). Furthermore, the PDGFs increase the proliferation and migration of synthetic smooth muscle cells [232,233].
ADOPTING ENDOTHELIAL-TO-MESENCHYMAL TRANSDIFFERENTIATION IN VASCULAR TISSUE ENGINEERING

The process of EnMT described above is complex and involves multiple converging signaling cascades. However, it becomes evident that the primary motor is TGF-β aided by the PDGFs. We therefore investigated if the mere addition of these factors to EC cultures would transdifferentiate endothelial cells into functional SMC. In a recent report, we show that EC indeed can be transdifferentiated into functional SMC by sole stimulation with TGF-β and PDGF-BB [165]. Furthermore, current experiments in our lab using EPC show similar results. EPC efficiently transdifferentiate into contractile SMC after stimulation with TGF-β and PDGF-BB. Moreover, transdifferentiation of EPC had no impact on their longevity, since telomerase activity was unchanged throughout the EnMT process (unpublished data). Likewise, Miyata and coworkers showed efficient transdifferentiation of the immortalized EPC cell line TR-BME2 after stimulation with PDGF-BB [234]. Thus, a functional link can be established between the EC and SMC lineages which can be utilized for vascular tissue engineering.

Although differentiation of both EC and SMC from a single progenitor cell is a huge step, herewith a blood vessel is not created. Besides EC and SMC, a natural blood vessel contains a basement membrane and an extracellular matrix which provides strength to the vascular conduit. In vascular tissue engineering, these matrix components are mimicked by degradable biomaterials that provides the cells with a temporal structural support. Such biomaterials would have to be non-immunogenic, anti-thrombogenic and degraded at a similar rate at matrix formation by the cells it contains in order to maintain the mechanical properties of the vessel conduit throughout the remodeling phase (reviewed in [235]).

Also, the biomaterial must support the organization of cells across several layers. In a natural blood vessel EC and SMC occupy spatially distinct areas of the vessel. Such tissue architecture warrants the hemocompatibility of the vasculature by separating the thrombogenic SMC layer from the antithrombogenic EC layer. Hence, a biomaterial used for vascular tissue engineering must also be able to physically separate EC and SMC. This may be achieved by combining multiple materials or multiple material architectures as discussed below.

Finally, the correct instructions must be given to appropriately differentiate and position EPC. The differentiation of EC and SMC depends on different growth factors (VEGF and bFGF for EC and TGF-β and PDGF-BB for SMC) and occurs on different ECM components. Mimicking these ECM in a growth factor-releasing biomaterial would therefore greatly benefit the differentiation and organization of EC and SMC from a common progenitor cell and poses a huge opportunity for vascular tissue engineering. Hence, the next engineering challenge will be to tailor degradable biomaterial scaffolds to meet the developmental demands of endothelial cell differentiation and EnMT.

THE BIOMATERIAL CHALLENGE

Recent advances in biomaterials research highlight the importance of integrating biological and chemical science. Developmental biology has provided the determinants of progenitor cell differentiation, i.e. tissue architecture, progenitor cell plasticity and cell signaling through soluble mediators, while biomaterials research has provided the tools to integrate this biological knowledge into a new class of ‘smart biomaterials’ by investigating scaffold architecture, protein release and peptide-mimicry. These new biomaterials mimic the ECM, release growth factors and may meet the developmental demands of EC differentiation and EnMT. Therefore, the second part of this review focuses on these developments in biomaterial design.

From a chemical viewpoint, the choice of the polymer as backbone for a degradable biomaterial provides design opportunities as well as limitations. This subject has been thoroughly reviewed previously [235;236] and we will therefore focus only on current developments that add to the integration of biological modalities in scaffold design. These biological modalities include: (1) resemblance to the native tissue architecture and mimicking of tissue strength, (2) allowing cell adhesion, and (3) instructing cell differentiation or maintenance. Although distinct biomaterials have been developed for every distinct biological modality, the current biomaterial challenge is to combine these biological moieties.

‘SMART BIOMATERIALS’: INSTRUCTING ENDOTHELIAL CELL DIFFERENTIATION AND ENMT

Whereas classically biomaterials were used as (temporal) structural supports, novel smart biomaterials incorporate the principles of developmental biology. In principle, smart biomaterials are equipped with molecular clues mimicking certain aspects of tissue structure, extracellular environment, or a combination of both.

As described above, the native blood vessels contain distinct and separated compartments for both the EC and SMC; the endothelium adheres to the endocardial basement membrane, which is a densely packed structure. The endothelial basement membrane contains amongst others collagen type IV and laminin, with small and dispersed interconnecting pores (~20-40 nm in diameter) [237]. Therefore, the basement membrane basically resembles a thin film. In contrast, SMC are surrounded by the ECM, which mainly consists of fibrillar collagens that form a open porous structure (~50-200 μm pore diameter) [238] that basically resembles a sponge.

SCAFFOLDS MIMICKING TISSUE ARCHITECTURE.

Blood vessels obtain their strength and compliance from both the spatial organization of SMC and the aligned organization of their ECM [239]. Several groups have successfully attempted to recreate this spatial organization by using microtextured biomaterial templates that control cell organization and possibly matrix deposition [240;241]. Of particular interest is the work of Sarkar and coworkers, who showed high percentages of SMC alignment on microtextured solid polymers. In a follow-up paper, Sarkar and coworkers transferred their micropatterning technique from solid polymers to polymeric sponges by integrating soft lithography, melt molding and particle
leaching techniques [240]. As with the solid polymers, SMC showed high structural alignment in the polymer sponge.

Also, current developments in the electrospinning process have enabled the creation of biomaterials with aligned fibers [242:243], thus introducing ‘morphological smartness’ to biomaterials. In due time, also other instructive clues will also be incorporated in these aligned biomaterials. These may be ECM-mimics, driving cell adhesion and migration, or growth factors, driving differentiation and proliferation, but, one can hope, a combination of the two as discussed below (Figure 3A).

**Scaffolds Mimicking the Extracellular Matrix.**

Beyond the physical and architectural properties of polymers (reviewed in [244], a major goal in ‘smart material design’ is to integrate biomaterials and the specific properties of signaling mediators that dictate cell behavior, such as ECM components and growth factors. Combining the morphological ‘smartness’ (described above) with instructive signals would allow for the simultaneous differentiation of EPC into EC as well as SMC in a single material, and thus the development of highly organized vascular conduits (Figure 3).

One of such developments lies in protein mimicry. Small synthetic peptides can be used to mimic the integrin-binding-sites of ECM molecules. Examples are synthetic mimics of fibronectin (RGD and PHSRN) [245], laminin (YIGSR and IKVAV) [246] or collagens (type I: DGEA; type IV: TAGSCLRKFSTM) [247:248]. The fibronectin mimics RGD and PHSRN have both been implicated in the differentiation of cells towards the endothelial lineage [249]; Alobaid and coworkers showed increased attachment and increased endothelial outgrowth when EPC were cultured on RGD-coated wells plates [250] and Fereira and coworkers reported a 20-fold increase in EC differentiation from embryonic stem cells, when these cells were encapsulated in a dextran-based hydrogel containing the RGD-peptide [251]. Of further interest, endothelial cells that were cultured on a mixture of the fibronectin-mimic RGD and the laminin-mimic YIGSR showed increased cell attachment and increased capillary tube formation when compared to scrambled peptide controls [252], indicative of synergy between multiple ECM mimics.

In contrast to the fibronectin mimics, the collagen mimic DGEA was shown to interact with the integrin αvβ3, found on SMC [253] and not with EC. DGEA-coating increased SMC spreading and aided the shift in phenotype from contractile to synthetic SMC [247].

From the above, we can conclude that the incorporation of small peptide sequences enhances the interaction between cells and biomaterials. Moreover, in varying the peptide sequences incorporated in the biomaterial, a separation may be achieved between several cell types (Figure 3A); that is an outer SMC layer growing on collagen type I mimics, and an inner EC layer on fibronectin/laminin mimics.

**Protein Releasing Scaffolds.**

The natural ECM modulates tissue dynamics by the ability to bind, store and release bioactive molecules like growth factors and cytokines. Therefore, the natural ECM acts like a release platform which is able to store and release a multitude of growth factors that in concert dictate cell behavior. As most cellular processes require more than one factor, recent research activities have been focusing on the sequential delivery of multiple growth factors, mimicking this ECM ability [254].

Heparin-modification of biomaterials is a well known method to add bioactivity to a scaffold. Heparins are well known for their ability to bind a variety of growth factors such as VEGF and basic FGF. Various groups have described the use of biomaterial-bound heparins for the release of one or multiple growth factors and their effect on cell maturation, the tissue engineered vessel is implanted into the patient.
Yet another interesting development was described by Gong and coworkers. They combined the mechanical properties of a porous scaffold with an instructive hydrogel that promoted the growth of chondrocytes. This straightforward approach resulted in a scaffold/hydrogel hybrid that has high mechanical strength and the ability to release biologically active modulators [257].

Although the methods above provide scaffolds that can supply their environment with growth factors and thus influence cell behavior, they are insensitive to that same environment. This renders the release of growth factors and tissue generation unsynchronized and may lead to side effects such as tissue overgrowth. Recently, new growth factor releasing biomaterials have been designed that incorporate another aspect of nature's smartness and can respond to their direct environment. Hubbel and colleagues have generated peptide sequences which are vulnerable to MMP-2-mediated or plasmin-mediated degradation [258;259]. Cross-linking these peptide sequences to growth factors and incorporation into biomaterials offers a platform for cell-dependent growth factor release [238]. In extrapolation, if various peptides would be incorporated into a biomaterial, which react to distinct cell-specific enzymes, that biomaterial may control cell differentiation in a spatially or temporally refined manner.

Taken together, the recent developments in 'smart biomaterial' design are leaning towards the incorporation of 'biological smartness' in polymeric biomaterials. Although currently most studies have focused on the incorporation of just one type of instructive signal, future developments will undoubtedly focus on combining instructive signals. Hence, biomaterials can be generated that on their luminal side release factors that promote EC differentiation and growth, while on their outer surface they release factors that promote SMC differentiation or EnMT (Figure 3A). Such 'smart biomaterials' will offer huge opportunities for vascular tissue engineering in the near future.

**DISCUSSION AND FUTURE ASPECTS**

The current challenge in vascular tissue engineering is to combine state-of-art knowledge on developmental biology and biomaterial science into a new tissue engineering paradigm. From developmental biology, knowledge on EPC differentiation and plasticity has led to new possibilities, i.e. the differentiation of proliferating EC and SMC from one single progenitor cell type, the EPC. Advances in biomaterial research have embedded molecular clues into a new generation biomaterials which act instructively on the cells they carry. The current challenge for tissue engineers is to adapt the current advances of both research fields in order to generate a new generation, self-assembling tissue engineered blood vessel.

An unexposed issue which remains in vascular tissue engineering is the availability of EPC. We started this review by shortly describing how EPC and their progeny EC can be isolated and transdifferentiated into SMC by the addition of TGF-β and PDGF-BB. Subsequently, we illustrated how developments in the field of biomaterial research can be applied to tissue engineer a progenitor cell-based small-diameter blood vessel (Figure 3). However, the numbers and function of these EPC may be affected as a result of disease. We and others have described functional impairment of endothelial progenitor cells in various diseases, including inflammatory diseases [108], renal diseases [260] and cardiovascular diseases [261]. These impairments pose a new challenge for progenitor cell-based tissue engineering and strategies to overcome these impairments have to be addressed in future research.

Also, recent data indicates that EPC do not only function in tissue engineering by differentiating into EC and SMC, but also through paracrine signaling [262;263]. EPC were shown to induce angiogenesis by the secretion of, amongst others, VEGFα and basic FGF [113], but also secrete IL-1β and TNFα [264]. Therefore, one could anticipate that, also EPC in a tissue engineered blood vessel secrete such factors and thereby affect the neighboring cells. Whether such paracrine interaction between a tissue engineered blood vessel and the native tissue is beneficial, remains to be addressed in future research.

**CONCLUSION**

In conclusion, advances in the field of developmental biology and biomaterial science are providing new opportunities for the development of highly organized tissues. Here we have discussed the use of a single progenitor cell type, the EPC, for the generation of a small-diameter blood vessel. In the approach provided, the differentiation of these EPC towards the vascular cells that built up the vessel is completely driven by bioactive modalities within a degradable biomaterial. The application of such 'smart biomaterials', that use molecular clues to direct and modulate (progenitor) cell differentiation, will reduce laborious and costly culturing protocols and will enable tissue engineers to make a great leap towards functional restoration of blood flow.
REFERENCES


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