DOI: 10.1002/macp.201600574

Article Type: Talents

Promoting Endothelialization of Polymeric Cardiovascular Biomaterials

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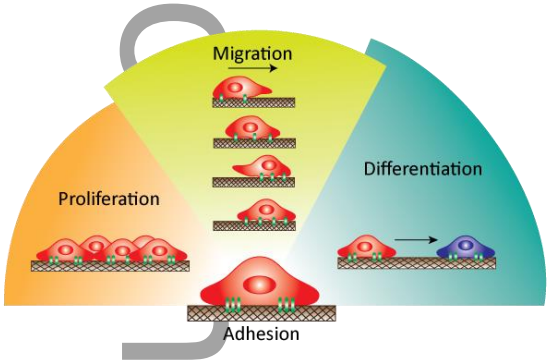
The lack of a blood compatible synthetic interface is one of the largest unaddressed challenges in the field of biomaterials science. This technological shortcoming hinders the successful clinical application of small diameter vascular grafts and other cardiovascular devices such as stents and artificial heart valves. Therefore, intensive research activities are ongoing to develop polymer materials with improved blood compatibility. One attractive strategy to improve the blood compatibility of an interface is to design surfaces that promote the development of an endothelium, the monolayer of endothelial cells that line our native vasculature and is responsible for blood compatibility. This article describes the recent strategies that have been used to generate polymeric materials that promote the development of an endothelium, discusses shortcomings in the field, and proposes future directions of research that can be undertaken to design next generation polymeric biomaterial that promote endothelialization.



1. Introduction

Cardiovascular biomedical devices are well-established tools in today's medical landscape and include a wide range of lifesaving products such as artificial vascular grafts, heart valves, pacemakers, etc.[1,2] Despite the success of these devices, materials-related problems still exist in this arena. Specifically, many of the commercially available devices are made from commodity materials that interact in undesirable ways with the biological environment. A chief problem in the cardiovascular device field is the lack of blood compatibility. Coagulation occurs when the device is exposed to blood, resulting in the accumulation of a blood clot, or thrombus, on the device surface. In certain scenarios the thrombus can directly impair the function of the device, or a piece of the thrombus can embolize and cause life-threatening problems downstream such as stroke.[1,2]

One major shortcoming that arises from the lack of a blood compatible surface is the inability to produce a small diameter vascular graft to treat coronary artery disease (CAD) or lower leg ischemia. Thrombus accumulation occurs on all vascular grafts, but the presence of the thrombus does not impair the function of grafts with large diameters (≈7mm or greater). However, the accumulation of thrombus on small diameter vascular grafts is sufficient enough to impair device function, and vessels that feed blood to the heart and lower leg are often small diameter in calibre.[3,4] This makes treatment of CAD particularly difficult. CAD is caused by narrowing of the blood vessels that oxygenate the heart, usually due to atherosclerosis. In cases where less than 70% of the cross sectional area of the vessel is blocked, angioplasty followed by stenting is commonly used to restore blood flow. In cases where more than 70% of the cross sectional area of the vessel is blocked, bypass surgery is usually performed. Commercially available vascular grafts are made from commodity materials and fabricated to contain microporosity, usually expanded poly(tetrafluoroethylene) (ePTFE) or polyethylene terephthalate (PET). However, they are unsuitable for small diameter vascular graft applications as they clot far too easily.[1,2]

Bypass surgery using an autologous vessel is usually the necessary treatment under these conditions. However, this requires a vessel to be harvested from elsewhere in the patient and to be re-attached in order to route blood around the obstruction. Bypass surgery faces many challenges including second site morbidity, the lack of appropriate donor vessels in some patients, and sub-optimal performance of the grafted vessel due to its different anatomical point of origin and physiological purpose.[1,2] The development of a synthetic material with improved blood compatibility would enable the fabrication of small diameter vascular grafts that remain patent, thus filling this critical void in the cardiovascular device space.

Many methods have been pursued to improve the blood compatibility of blood-contacting biomaterials. Such efforts include using surfaces that display non-fouling chemical groups such as polyethylene glycol (PEG), zwitterionic groups, or polyoxazolines, or pre-coating the surface with the non-cell adhesive protein albumin in order to prevent the protein and platelet deposition that results in thrombus formation.[5–8] Other strategies include the immobilization of anticoagulants to the graft surface, most notably heparin.[9–12] While many of these strategies have shown improved blood compatibility in vitro, in vivo success still remains elusive.

The native blood vessel consists of three layers, or tunica, each of which is critical to the function of the blood vessel. In this article, we are particularly interested in the inner most layer, the tunica intima, which consists of a confluent monolayer of endothelial cells (ECs) that are in their quiescent state. The monolayer of quiescent ECs performs several key biological functions including the production of anticoagulant factors that actively prevent thrombus formation.[13,14] Therefore, creating materials systems that actively foster a confluent and functioning endothelium is an attractive strategy for generating a blood compatible interface. This article specifically focuses on the design, synthesis, and fabrication of polymeric devices designed to promote endothelialization for use in cardiovascular devices.

2. Mechanisms of In Vivo Endothelialization

Three main mechanisms exist by which endothelialization occurs in vivo: transanastomotic growth, transmural growth, and scavenging of blood-borne cells (**Figure 1**).[15–17] When a vascular graft is implanted, it must be attached to a native vessel at its end points. These points of attachment are called the anastomoses. At these end points, mature endothelial cells can migrate from the native vessel onto the luminal surface of the graft. This is referred to as transanastomotic growth. This growth is significant in some animals; however, transanastomotic growth only extends 1-2 cm from the anastomoses in humans meaning it is not sufficient to endothelialize most grafts, especially those of significant length.[2,15]

Most grafts are also fabricated to contain microporosity. Immediately after implantation, these grafts will ooze blood, the blood will clot in the interstitial space, and this clot will eventually be reorganized and allow tissue ingrowth and integration. This microporosity also

enables the possibility of endothelialization through transmural growth. In this instance, the tissue that grows into the interstitial space of the graft will be vascularized, these vessels can reach the lumen of the graft, and endothelialization can occur at these points.[2,15]

The third modality by which endothelialization can occur is through blood-borne cells. In recent years, cells in circulation that have the capacity to differentiate into cells of endothelial phenotype have been identified. These cells are referred to as endothelial progenitor cells (EPCs) and will be described more fully in the following section. It is possible that these EPCs will adhere to the surface of the graft, differentiate, proliferate, and lead to islands of endothelium.[2,17] However, these mechanisms are insufficient to successfully endothelialize a vascular graft in human.

3. Sources of Endothelial Cells

Endothelialization has been attempted by implanting bare grafts in the hope that in situ endothelialization will occur or by pre-seeding the graft with endothelial cells prior to implantation. Either scenario requires a source of autologous cells with endothelial phenotype, of which there are two main sources (Figure 2). In situ endothelialization strategies aim to achieve endothelialization through the mechanisms mentioned described in Figure 1 (transanastomotic growth, transmural growth, or adhesion of blood-borne cells), while pre-seeding of the graft with endothelial cells requires isolation and *ex vivo* expansion of autologous cells prior to the implantation. Using mature endothelial cells to pre-seed a graft is challenging as it requires the biopsy of vascular tissue, the isolation of the ECs, and the ex vivo expansion of these cells to therapeutically relevant cell numbers.[18,19]

In recent years a more attractive strategy has emerged. In 1997, Asahara, et al. identified endothelial progenitor cells (EPCs), mononuclear cells present in adult human peripheral blood that give rise to cells with endothelial phenotype during *ex vivo* culture.[20] EPCs are phenotypically distinct from mature ECs and are present in very low frequencies in peripheral blood; however, they are advantageous over mature endothelial cells in that they can be collected through a simple blood draw.

The EPC population is heterogeneous, and when these cells are cultured under appropriate conditions *ex vivo* they give rise to three distinct populations of endothelial-like cells: colony forming unit-Hill cells (CFU-Hill cells), circulating angiogenic cells (CACs), and endothelial

colony forming cells (ECFCs) which are also referred to as blood outgrowth endothelial cells (BOECs).[21–25] CFU-Hill cells display certain cell surface markers that are consistent with endothelial cells, but they also exhibit surface markers consistent with monocytes/macrophages. Current evidence points to the ideas that these cells may promote angiogenesis, but may not become part of the endothelium in vivo. Similarly, CACs exhibit expression of certain endothelial markers; however, they do not exhibit robust proliferation and colony formation in culture. The last population, the ECFCs, appear after 2 – 3 weeks in culture and are phenotypically similar to ECs. Additionally, these cells exhibit robust proliferative capacity, enabling the generation of a large number of highly proliferative cells with endothelial phenotype.[21–25] Thus, of the three populations of cells that can be isolated from EPCs through *ex vivo* expansion, the ECFCs have received the most interest from researchers interested in the endothelialization of biomedical implants.

4. Polymeric Biomaterials that Promote Endothelialization

Autologous endothelialization appears to be one of the most promising strategies for the development of blood compatible interfaces. As a result, a wide array of research has been performed to develop polymeric materials that promote endothelialization, and these strategies are the major focus of this article. Specifically, we will look at how fundamental polymer properties such as stiffness affects endothelialization, assess how fabrication techniques can be used to generate favourable surface topographies for endothelialization, and finally address various biofunctionalization strategies that can be utilized to generate surfaces that promote endothelialization (**Figure 3**).

4.1. Substrate Stiffness

A second major technological challenge that limits the use of small diameter vascular grafts is neointimal hyperplasia.[26] Current vascular grafts are made of materials that are much stiffer than native vasculature. This compliance mismatch is believed to result in excessive proliferation of smooth muscle cells present in the walls of the vasculature due to altered suture line stresses and damaging changes to the hemodynamic forces experienced by the endothelium near the anastomoses.[27–29] This over proliferation of the smooth muscle cells

can also results in narrowing of the luminal area and failure of the graft. One method of addressing this issue is generating devices that elute antiproliferative drugs.[30,31] However, the local reservoir of drugs is limited, and problems can arise once the drug depot is depleted. Two strategies that are more promising are the development of materials that exhibit similar mechanical properties to the native vasculature and the development of materials that foster a functioning endothelium.[32,33] Alleviating the mechanical mismatch between tissue and graft will minimize the driving force for neointimal hyperplasia. Additionally, a functioning endothelium shields the underlying smooth muscle cells from factors in the blood stream and locally produces antiproliferative agents.[14] In recent years, polyurethane grafts have shown promise in this space due to their more acceptable mechanical properties when compared to native vasculature.[34,35]

4.2. Substrate Topography

In vivo, many cells are adherent to an extracellular matrix (ECM), a highly hydrated fibrous network of biomolecules. The fibers that comprise the ECM are smaller in diameter than the diameter of the cells, and the ECM provides many important functions such as mechanical support for the adherent cells, promoting cellular orientation, and maintenance of cellular phenotype.[36,37] Generating polymeric scaffolds that replicate the nanometer-to-micron-scale of the ECM is a proven method for modulating the behaviour of adherent cells, and a wide variety of scaffold fabrication techniques have been developed including particle leaching, phase inversion, micromolding, etching, cryogelation, electrospinning and others.[5,6,38–44] Substrate topography has been shown to be a key regulator of many critical endothelial cell behaviours including adhesion, morphology, migration, proliferation, and gene expression during in vitro culture.[45–50] One surface pattern that has shed valuable insight into endothelial cell behaviour is parallel microgrooves. These surfaces are fabricated to contain a repeating pattern of parallel microgrooves where the size of the groove depth, groove width, and distance between grooves can be easily controlled from the nanometer to micron-scale. When endothelial cells are seeded onto these surfaces their behaviour is modulated by the surface topography. Franco, et al. illustrated that appropriate microgroove structures promoted endothelial cell adhesion. Specifically, the researchers generated aligned microgrooves on olefin surfaces using imprint lithography and found that certain topographies increased the spreading of ECs by 40%. The acceleration of spreading was

attributed to faster activation of adhesion-dependent signalling within the cell.[49] Lu, et al. also showed that initial endothelial cell adhesion is more rapid on surfaces patterned with microgrooves. Additionally, these surfaces also contained larger numbers of cells after one day of culture, and the endothelium reached confluence at an earlier time point illustrating increased proliferation rate of the ECs.[47] Ding, et al. generated a series of surfaces that were patterned with microgrooves and were also covalently immobilized with heparin. The researchers also showed that surfaces patterned with microgrooves promote endothelial cell adhesion and proliferation. Additionally, they illustrated that these surfaces showed specificity towards the adhesion of endothelial cells over smooth muscle cells, though this specificity was due more to the heparin coating than the microtopography.[48] These microgrooved also promote elongation of the cells in the direction parallel to the grooves. In vivo, the ECs are also aligned in the direction of blood flow, thus this microtopography can influence the adherent endothelial cells and result in morphology more similar to what is observed in vivo.

Potthoff, et al. aimed to identify the optimal size of these grooves in promoting endothelialization. The previous studies have illustrated that ECs exhibit optimum response on grooves that are 1 μm in width. However, the optimum groove depth remains obscure. In this work, the researchers explored grooves with widths of 1 μm and depths ranging from 100–1000 nm. The researchers identified that deep grooves (1 μm) resulted in enhanced signalling activity through more activation of focal adhesion kinase while shallower grooves (100–400 nm) improved the adhesion strength of the cells to the surface.[50] Jeon, et al. also assessed how these microgroove topographies influenced EC secretion of cytokines and chemokines. In addition to observing elongation of the endothelial cells in the direction of the grooves, the presence of the topography also resulted in a decrease in the secretion of inflammatory cytokines.[45] These results indicate that surfaces fabricated to contain parallel microgrooves may be more suitable for endothelialization compared to flat surfaces of the same material, and that the dimensions of the microgrooves can be tailored to promote specific EC behaviours.

Although the microgrooves described above elicit many desirable EC behaviours, the fabrication of these surfaces requires sophisticated and expensive instrumentation. Electrospinning has recently emerged as a power technique for the fabrication of nonwoven fibrous scaffolds comprised of fibers of micro- or nano-scale diameters. The process is particularly appealing as a laboratory-based process as it is a low cost and low temperature

fabrication technique that uses readily available equipment and does not require a clean room.[51,52] Additionally, the fibrous nature of the scaffolds closely resembles the cell's native environment, the ECM.[53] Standard electrospinning uses polymer solutions. The fibers are produced by perfusing the solution through a needle tip. A large voltage difference is applied between the needle tip and a grounded collector plate resulting in a thin jet of polymer solution flying to the collector plate. During the flight, the polymer jet dries, resulting in the deposition of non-woven and randomly oriented polymer fibers on the collector. Additionally, if the collector is rapidly rotating, the fibers can be drawn into alignment during deposition resulting in scaffolds of aligned fibers.[51,52,54] Many bio-stable and biodegradable synthetic polymers as well as a large number of biopolymers have been electrospun.[53] The electrospinning of polymer melts is also possible, but the solution-based technique is more extensively explored.[55]

Electrospun polymer scaffolds have also been shown to promote desired EC behaviours that may be useful when fabricating next generation vascular grafts.[44,54,56–59] Hadjizadeh, et al. illustrated that fiber diameter and orientation influence EC behaviour. Polyethylene terephthalate electrospun scaffolds were produced with two different average fiber diameters (740 nm or 1.8 μm) and orientations (low and high). ECs were able to adhere and proliferate on all scaffolds; however, the proliferation rate was higher for cells on scaffolds with larger fiber diameters. Additionally, the cells were able to penetrate into the scaffolds with the larger fiber diameters while they were confined to the surface of the smaller-diameter scaffolds. Additionally, the presence of fiber alignment resulted in improved alignment of the adherent ECs.[56] Ko, et al. also observed differences in EC behaviour due to the fiber size of the electrospun scaffolds. In this work, the researcher fabricated poly(lactic-*co*-glycolic acid) scaffolds with random fiber orientation over a range of fiber diameters (200 nm to 5 μm). Scaffolds with smaller fiber diameters resulted in faster initial cell adhesion; however, larger fiber diameters enabled cellular penetration into the scaffolds and resulted in higher degrees of proliferation after two weeks of culture.[57]

ECs migrate into electrospun scaffolds and then proliferate faster is an interesting observation. However, the author of this article doubts the use of these scaffolds in vascular graft applications. In vivo, ECs are present as a monolayer on the luminal surface of the blood vessel, while other cell types such as smooth muscle cells inhabit the walls of the tissue. Therefore, generating electrospun scaffolds with microporosity that allows infiltration of surrounding tissue after implantation, promotes the proliferation of ECs, and confines the

endothelium to a two dimensional monolayer on the interior surface of the graft would be of great interest. Previously, we synthesized a family of methacrylic terpolymers with a range of glass transition temperatures (T_gs). By specifying the glass transition temperature of the materials, we were also able to control the mechanical properties of the material at physiological conditions.[58] When these materials were electrospun into fibrous scaffolds, the T_g of the material affected architecture of the scaffold. Specifically, high T_g materials produced scaffolds with discrete fibers, large pores, and void space while low T_g materials produced scaffolds with fused fibers, smaller pores and significantly less void space.[54] When ECs were cultured on these surfaces, we observed that the low porosity scaffolds significantly promoted EC proliferation and spreading compared to the other scaffold types and planar surfaces of the same material and confined these ECs to a monolayer on the surface of the scaffold.[54]

The above experiments were all performed under static cell culture conditions. However, endothelial cells are constantly exposed to shear stress due to the flow of blood over their surface. Therefore, it is necessary to develop material systems that improve the adhesion strength of the cells to prevent their dislodgement under flow. Whited, et al. produced fibrous scaffolds from a blend of poly(ε-caprolactone) (PCL) and collagen type I with a range of average fiber diameters (100–1200 nm) and varying degrees of fiber alignment. ECs were able to adhere and proliferate on all surfaces, and cell orientation increased with increasing fiber orientation. Most importantly, the researchers illustrated that ECs on highly aligned scaffolds showed greater resistance to detachment due to flow compared to other surfaces.[59] These results illustrate that electrospinning is a useful technique to produce polymeric surfaces that can regulate key EC properties such as adhesion, morphology, proliferation, and adhesion strength under flow.

4.3. Surface (Bio)Chemistry

In addition to a material's stiffness and topography, the chemical nature of the interface plays a significant role in regulating cell-material interactions. When a biomaterial is exposed to blood, cell culture medium, or another solution of biomolecules, proteins from the solution phase will adsorb to the interface. This protein deposition is a thermodynamically driven process and the composition of the protein layer, the orientation of the proteins at the surface, and potential denaturation of the proteins is largely governed by the chemical properties of

the polymer interface.[60,61] The composition and conformation of the proteins at the interface then drive behaviours of cells that come into contact with the surface.[62,63] If a virgin biomaterial surface is placed in contact with a complex mixture of biomolecules, the researcher has little control over which biomolecules adhere and in what proportion. Thus it is common practice is to pre-coat a surface with a desired protein or blend the protein within the polymer matrix. Common proteins of choice are those naturally present in the ECM including collagen, fibronectin, and laminin.[64–68] In one study, Ino, et al. blended poly(vinyl alcohol) with gelatin and assessed the in vitro ability of the materials to develop an endothelium. It was found that ECs exhibited drastic improvements in their ability to adhere and proliferate on blends containing 1 weight % of gelatin and were able to develop a confluent endothelium. However, blends with higher weight percentages of gelatin (up to 10%) did not foster a confluent endothelium. The author's rationalized this observation by attributing it to phase segregation between the poly(vinyl alcohol) and gelatin and the formation of microdomains that inhibited EC adhesion and growth. Additionally, the incorporation of the gelatin altered the mechanical properties of the material.[66] For these reasons, the author of this article believes that surface modification strategies are a superior technique, especially for long-term implants that are meant to be biostable. For instance, Sgarioto, et al. illustrated that collagen-coated polystyrene significantly improved the proliferation of ECs in vitro. Additionally, the pre-adsorption or covalent attachment of ECM components to the surface has significantly improved the in vivo performance of vascular grafts.[64] Seeger, et al. implanted fibronectin-coated ePTFE grafts in dog models. Some grafts were also pre-seeded with ECs while others were not. The grafts that were coated with fibronectin and seeded with ECs exhibited the greatest extent of endothelialization followed by coated grafts that were not seeded with cells. Both of these treatments exhibited more endothelialization compared to plain grafts.[65] Williams, et al. covalently coupled laminin type 1 to the surface of ePTFE grafts and studied the in vivo endothelialization in a rat model. After 6 weeks, grafts were explanted, and the researchers observed improved endothelialization of the surfaces over controls as well as improved neovascularization of the interstices of the graft.[67] One limitation associated with coating of the graft with biomolecules is that platelets – the cells that are also responsible for thrombus formation – also bind to many of the proteins mentioned above. The ideal surface modification would promote the specific adhesion and growth of endothelial cells but not adhere platelets. Lord, et al. coated an ePTFE graft with the heparin sulphate proteoglycan perlecan and studied their

endothelialization in a sheep model. It was found that the perlecan-coated grafts simultaneously promoted endothelial cell adhesion and proliferation while minimizing platelet adhesion illustrating that this surface coating can promote the specific binding of endothelial cells.[68]

4.3.1. Surface Functionalization with Peptide Ligands

As described above, coating whole proteins to a surface provides a researcher with only limited control of the resulting surface characteristics. Therefore researchers have developed a variety of synthetic materials that aim to (partially) replicate the key biological properties of the ECM. Although the ECM proteins are of high molecular weight, cells often attach with high specificity through their integrin receptors to relatively short amino acid sequences present in the structure. The most notable example is the arginine-glycine-aspartic acid (RGD) tri-peptide unit present in ubiquitous ECM protein fibronectin.[69,70] When these short amino acid sequences are covalently incorporated into a polymeric biomaterial, researchers are rewarded with specific adhesion of cells that possess the appropriate integrin receptor for the given ligand. However, protein fouling is a barrier in this technology. The presence of the adsorbed protein layer can act to hide the ligands of interest from view of the adhering cells.[71] Therefore, the peptides are often immobilized to a non-fouling interface [usually poly(ethylene glycol)] that resist non-specific adsorption of proteins yet enable adhesion of cells that possess the appropriate integrin receptors.[43,72] Additionally, the generation of synthetic materials that bare cell-adhesive peptides on the surface provide a researcher with control over the surface density of the ligand, and this jurisdiction over surface density enables a researcher to regulate various aspects of adherent cell behaviour including adhesion, proliferation, migration, etc.[73–75] Many other cell adhesive ligands exist that interact with endothelial cells and that show varying degrees of specificity to other cell types. Most notably is the REDV peptide that is believed to be endothelial cell-specific.[76] Table 1 compiles several peptide ligands that have been used to promote endothelialization of biomaterials or that show significant angiogenic potential.

There are several limitations to this technology. The first is that the non-fouling polymers used to incorporate protein resistance to the interface (ex. PEG) operate through strong interactions with water molecules, thus adsorption of proteins is thermodynamically unfavourable.[5–8,60,61] As a result, the bulk materials that contain these groups are often

plasticized in an aqueous environment and do not possess appropriate mechanical properties. Therefore, crosslinking strategies or surface modifications of an otherwise hydrophobic polymer has been developed.[77–79] Additionally, the most common non-fouling polymer, PEG, undergoes degradation in the in vivo environment making it unsuitable for long-term biomedical implants. For these reasons alternative non-fouling polymers such as the zwitterionic materials or the polyoxazolines have been developed.[6,80–82]

Another shortcoming with this biofunctionalization strategy is that many integrin receptors are conserved across cell types. For instance, the RGD peptide binds to multiple integrin receptors, and these receptors are present on more than just endothelial cells. Most notably, platelets also possess the ability to bind to RGD-functionalizes surfaces. Therefore, developing strategies that enable a stable endothelium to be formed prior to implantation or the rapid development of an endothelium post-implantation is required. While most of the peptide ligands listed in Table 1 are derived from ECM proteins, several have been identified using phage display technology. Phage display is a combinatorial approach where a library of potentially billions or more peptide candidates are narrowed down through a series of positive and negative enrichment steps in order to find ligands that bind strongly and with high specificity to a certain target of interest. Previously, we screened a phage display library to find peptide ligands that bind with high specificity to BOECs but not other commonly occurring cell types or mature ECs.[83] Subsequent work has illustrated that the TPSLEQRTVYAK peptide is also resistant to platelet deposition. We then covalently incorporated these ligands into a synthetic biomaterial and showed specific adhesion of BOECs for the first time.[71] Since its identification, the TPSLEQRTVYAK-peptide has gained significant attention in the field of endothelialization.[84–87]

4.4. Shear Stress

A unique criterion that must be considered when designing materials for use in cardiovascular applications is shear stress. When the device is deployed, the surface of the device will be exposed to the hemodynamic conditions of blood flow, resulting in the presence of shear stress at the device interface. Many in vitro assays assess endothelial cell/material interactions under static conditions; however, the information collected from these experiments is incomplete. For instance, static culture experiments do not assess the adhesion strength of endothelial cells to the substrate. If the shear stress is large enough to

overcome an endothelial cell's adhesion strength, the cells may be sloughed off from the surface once exposed to flow.[88] This would result in denuding of the device, and exposure of the underlying surface that is potentially pro-thrombogenic. For vascular grafts that are pre-seeded with endothelial cells, it may beneficial to expose the cell-laden graft to shear stress prior to implantation. Using a rat model, Dardik, et al. observed improved endothelial cell retention and reduced intimal thickening when EC-seeded grafts were pre-conditions with shear stress prior to implantation.[89] Additionally, many researchers are currently focusing on endothelializing cardiovascular biomaterials by scavenging appropriate cells from circulation. In this situation, the binding strength between the circulating cell and the surface must be sufficiently strong and fast forming to halt and adhere the desired cells from flow. Recently, Wang and Cooper used a radial flow chamber to showed that outgrowths of EPCs had a superior ability to adhere to peptide-functionalized surfaces compared to HUVECs, over a range of shear rates.[72]

Additionally, the presence of shear stress is a critical modulator of endothelial cell behaviour.[90] Specifically, adherent ECs and EPCs exposed to shear have been reported to align in the direction of flow, exhibit increased migration, improved proliferation, and upregulation in secretion of transforming growth factor-beta 1 release and basic fibroblast growth factor.[91–95] Much of this mechanotransduction arises through integrin signalling. For instance, Tzima, et al. illustrated that integrin activation by shear stress mediates Rho-dependent cytoskeleton alignment; Yang, et al. showed that shear stress activates eNOS through □1 integrins; and Urbich, et al. identified that shear stress-induced endothelial cells migration involves the α5 and β1 integrins.[92,96,97] The observation that these shear-induced behaviours occur through activation of specific integrins is critical in biomaterials design. For instance, when generating a peptide-functionalized biomaterial surface, the use of appropriate ligands is required to ensure that the desirable behaviours occur through the binding of appropriate integrins.

Although shear stress can promote many beneficial EC behaviours, abnormal shear stress has also been implicated in a variety of cardiovascular diseases including arteriosclerosis and neo-intimal hyperplasia.[98] For instance, data suggests that native vasculature that experiences lower than normal values of wall shear stress promotes arteriosclerosis progression.[99] Similarly, alterations in wall shear stress that occur around stents correlates strongly with where neointimal hyperplasia may occur.[100] Taken together, these data illustrate that shear stress is a key regulator to endothelial cell function, and must be

accounted for in the design of new cardiovascular biomaterials. The author suggests that both static and dynamic in vitro experiments are performed when evaluating the response of endothelial cells to a biomaterial surface. Additionally, it may be beneficial to expose an EC-seeded device to shear stress prior to implantation, and the device must be fabricated in a way to minimize adverse alterations of shear stress to nearby tissue.

5. Conclusions

Despite the advancements that have been made in generating biomaterials that promote endothelialization, significant progress can still be achieved. The author feels that generating biomaterials that more faithfully recapitulate key features of the basement membrane, the ECM layer that ECs adhere to in vivo, is the most promising avenue for developing materials that fully endothelialize. Although the biofunctionalization strategies described above are a promising start, many critical aspects of the basement membrane are not included in these systems. Most of the peptides used in these systems bind to integrin receptors, and the presence of these peptides on the surface is enough to result in integrin-mediated cell adhesion. However, integrins are also signalling molecules, and integrin-mediated signalling is critical in promoting cell-specific phenotype. But binding of an integrin to a ligand is not sufficient to initiate these signalling events. Biology research has shown that both integrin occupancy by a ligand and clustering of the integrins within the cell membrane are required before integrin-mediated signalling occurs.[101] Researchers have developed a variety of nanomaterials that promote both integrin occupancy and clustering in order to initiate intracellular signalling and improve a wide variety of cellular behaviours including adhesion, proliferation, migration, and differentiation.[102–104] However, these strategies have not yet been used to promote endothelialization of cardiovascular biomaterials. Additionally, a large number of studies focus on designing materials that bind integrin receptors. However, there are other types of adhesion receptors present on the cell surface, and it has been illustrated that there is significant crosstalk between integrins and these co-receptors.[105–108] Generating materials that engage a wider variety of cell adhesive molecules holds the potential of improving endothelialization. Additionally, the ECM the ECM serves many other functions beyond providing adhesion sites, structure, and mechanical anchoring for cells. Other ECM components (proteoglycans and glycoproteins) also serve as a local reservoir for endogenously produced growth factors and enhances the activity and availability of these

signalling molecules.[68,109–112] The author feels that generating new polymeric biomaterials that mimic these features of the ECM hold promise in promoting endothelialization and blood compatibility of future cardiovascular devices.

Acknowledgements

The author would like to acknowledge the University of Melbourne, the Therapeutic Technologies Research Initiative, and Equity Trustees for financial support.

Received: December 12, 2016; Revised: January 16, 2017; Published online: DOI: 10.1002/macp.201600574

Keywords

blood outgrowth endothelial cell; endothelial cell; endothelial progenitor cell; small diameter vascular graft; thrombus

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Figures

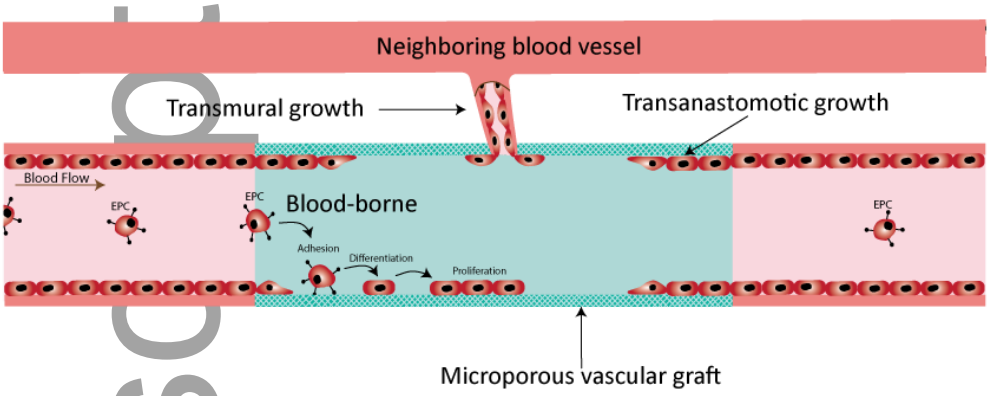


Figure 1. The three main mechanisms of the in vivo endothelialization of microporous vascular grafts are transanastomotic growth of endothelial cells from the end points of the graft, transmural growth of endothelial cells from neighbouring tissue/vessels that grow through the microporosity of the graft, and blood-borne adhesion and subsequent proliferation of endothelial cells or their precursors. **qry au:** If you have not returned the color cost confirmation form already, please email the completed form to the editorial office when you submit your proof corrections. This will confirm that you are willing to support the cost for color publication of the figures. Details about our color policies and a link to the form were included with your acceptance email. If you wish for your figures to be presented in greyscale, please email the editorial office to confirm this.

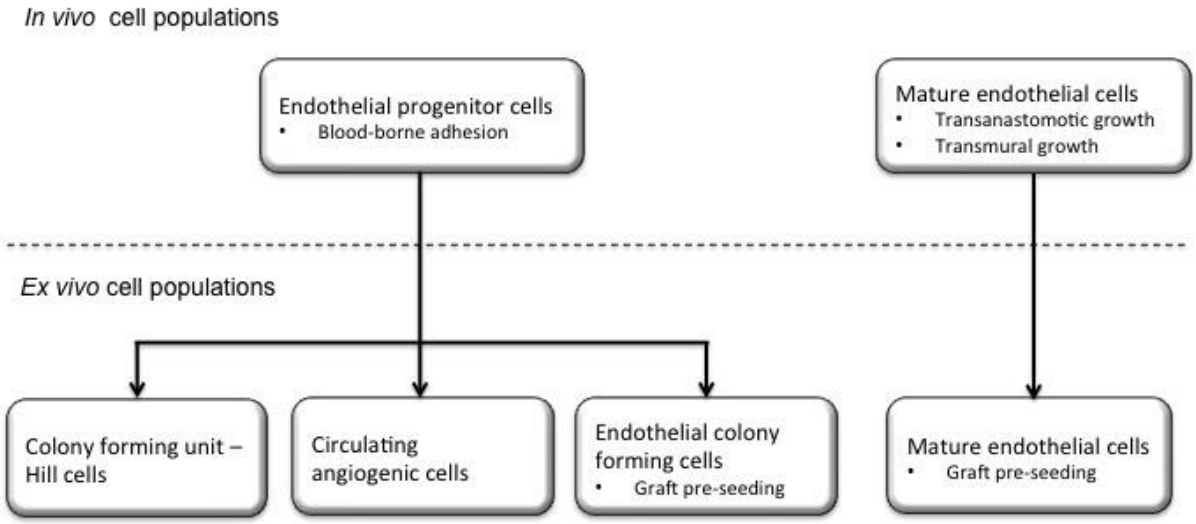


Figure 2. Cell types that may be useful in the endothelialization of polymeric biomaterials. Some cell types are present in the in vivo environment (ECs and EPCs) and can participate in in situ endothelialization. Other cells types can be isolated during culture (specifically ECs and ECFCs) and can be used in the pre-seeding of the graft prior to implantation. **qry au:** Could you please provide this figure in higher resolution?

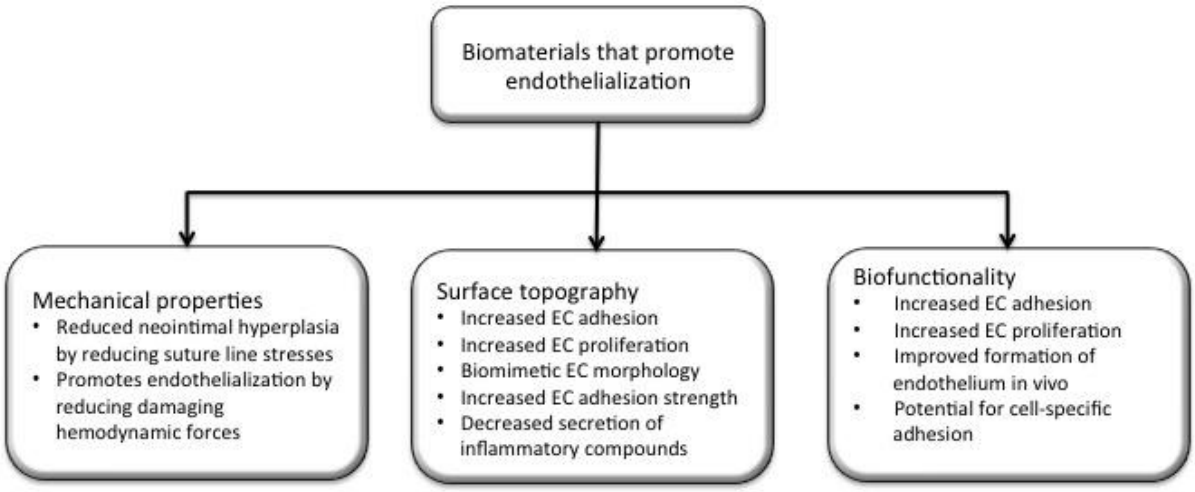


Figure 3. Different materials-based strategies that can be used to improve the patency rate of cardiovascular devices through improved endothelialization and reduced neointimal hyperplasia. Mechanisms include tailoring the mechanical properties of the polymeric material, fabricating the devices with appropriate micro/nano-topography, and biofunctionalization strategies. **qry au:** Could you please provide this figure in higher resolution?

Tables

Table 1. Cell adhesive peptide sequences that have been incorporated into biomaterials that promote adhesion of endothelial cells or improve angiogenesis. The CD34+ cells are a population of cells in circulation that contain EPCs.

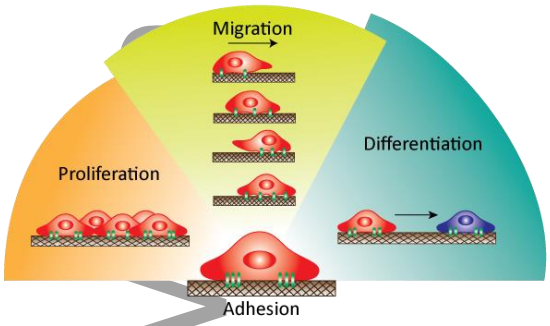
Peptide	In Vitro/In Vivo	Cell Type	Reference
Fibronectin derived			
RGD	in vitro	ECs and BOECs	[43,71,113]
WQPPRARI	in vitro	ECs	[114,115]
EILDVPST	in vitro	CD34+ cells	[116]
REDV	in vitro	ECs	[76,117]
Collagen derived			
P15 (GTPGPQGIAGQRGVV)	in vivo	unknown	[118]
Laminin-derived			
YIGSR	in vitro	ECs	[119]
IKVAV	in vitro	ECs	[120,121]
AG73 (RKRLQVQSIRT)	in vitro and in vivo	ECs	[122]
Phage display-derived			
CRRETAWAC	in vitro	ECs	[123]
TPSLEQRTVYAK	in vitro	BOECs	[43,71,72]
GHMDMSPHAVID	in vitro	BOECs	[43,71,72]

For Table of Contents

Blood compatibility remains one of the most pressing challenges in the biomaterials field. Developing biomaterials that foster a confluent and functional endothelium is one of the most promising strategies for addressing this challenge. In this article methods that have been explored for promoting the endothelialization of biomaterials and proposed future research directions are described.

Promoting Endothelialization of Polymeric Cardiovascular Biomaterials

D. E. Heath



About the author

Daniel Heath received his Bachelors Degree in Chemical Engineering from Florida Institute of Technology. He completed his doctoral work in Chemical and Biomolecular Engineering with Professor Stuart L. Cooper at The Ohio State University. He undertook a postdoctoral position with the Singapore-MIT Alliance for Research and Technology (SMART) beginning in 2011. This position was co-advised by Professors Linda G. Griffith (MIT), Paula T. Hammond (MIT), and Mary B. Chan-Park (Nanyang Technological University). He began a Lecturer position in the Chemical and Biomolecular Engineering Department at University of Melbourne in 2014. His lab currently focuses on improving the blood compatibility of cardiovascular biomaterials, the development of biodegradable coronary artery stents, and the improvement of *ex vivo* stem cell expansion.