Advancements in Surface Modification Strategies of Blood-contacting Biomaterials to Improve Biocompatibility and Tissue Integration

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Declarations of interest: none

Acknowledgements

This work was supported by an NSERC Discovery grant and an Ontario Early Researcher Award to T.F.D.T.F.D is Tier II Canada Research Chair. JIW holds the Canada Research Chair (Tier I) in Thrombosis and the Heart and Stroke Foundation J. F. Mustard Chair in Cardiovascular Research

Abstract

Improving the performance of blood-contacting medical implants is a global health necessity aimed at reducing mortality and morbidity in patients with cardiovascular diseases. Surface modification of the biomaterials from which the implants are constructed has been used to reduce the risk of complications such as thrombosis and infection. Herein with a focus on vascular tissue engineering, we provide an overview of (a) fundamental hemodynamic considerations for blood-contacting biomaterials, (b) surface modification strategies to attenuate nonspecific adhesion of proteins, improve hemocompatibility, and induce the formation of a confluent endothelial lining, and (c) the guidelines for the clinical development of surface modified biomaterials.

Keywords: blood-contacting implants, thrombogenicity, hemocompatibility, surface coatings, lubricant-infused coatings

Introduction

Accounting for 17.7 million deaths per year and 23.6 million deaths by 2030, cardiovascular disease (CVD) is the leading cause of death worldwide, surpassing cancer and other diseases.[1,2] In 2020, CVD incurred a financial burden of $22.2 billion in Canada; an amount that is expected to increase alongside sociodemographic characteristics such as ageing of the population, urbanisation, and socioeconomic status, as well as environmental risk factors such as air pollution.[3] To address this urgent global health problem, vascular grafts have proven instrumental in enhancing the quality of life in patients with CVD and are widely used to reconstruct, replace, or bypass occluded vessels. Nonetheless, adverse events such as thrombotic occlusion, calcification, and stenosis occur and often lead to device failure.[4] Moreover, the use of synthetic graft materials in pediatric patients make future re-intervention inevitable due to their lack of growth potential.[4,5] Thus, implementing strategies to improve the biocompatibility and tissue integration of vascular grafts is of great importance.

Strategies to design vascular grafts with improved biocompatibility must be based on a thorough understanding of the molecular mechanisms that drive the host response to foreign bodies. Thus, the implantation of synthetic vascular grafts perturbs the homeostatic balance and triggers thrombosis and inflammation, which can lead to thrombotic occlusion, fibrous encapsulation, and clacification.[1,6] Surface modifications are aimed at avoiding these complications,[1] while recapitulating the mechanical properties of the native vessels. Synthetic blood vessels must be sufficiently elastic and compliant to prevent mechanical mismatch and resistance to long-term fatigue. In addition, they must have sufficient suture retention strength to tolerate the hydrodynamic and mechanical forces at the sites of anastomosis.[7,8]

Expanded polytetrafluoroethylene (ePTFE) and polyethylene terephthalate vascular grafts are those most widely used, but at least 50% fail within 10 years mainly because they lack a functional endothelium that resists thrombosis.[3,9] Surface modifications can reduce thrombogenicity, enhance patency, and promote tissue regeneration.[1,5,9] Reducing thrombogenicity through surface modification also can eliminate the need for antithrombotic drugs, thereby reducing the risk of hemorrhagic complications.[10,11] This review focuses on biomaterial surface modification techniques in current use in vascular tissue engineering.

2. Hemocompatibility of Artificial Vascular Grafts

Sustained hemocompatibility of implants is critical for their long-term success. Since implants are recognized as foreign objects by the body, adverse interactions between the implant and human blood need to be analyzed to ensure that there is little or no activation of the blood coagulation and inflammatory systems.[3,12] Inadequate control of these adverse interactions can trigger interconnected pathological processes that results in thrombosis, hemodynamic instability, bleeding complications, and organ damage (Figure 1a). Such responses primarily depend on the surface properties of the artificial vascular materials such as pore size (Figure 1c, d), implantation site, and those of interacting proteins, cells, and neighboring blood flow.[5,13]

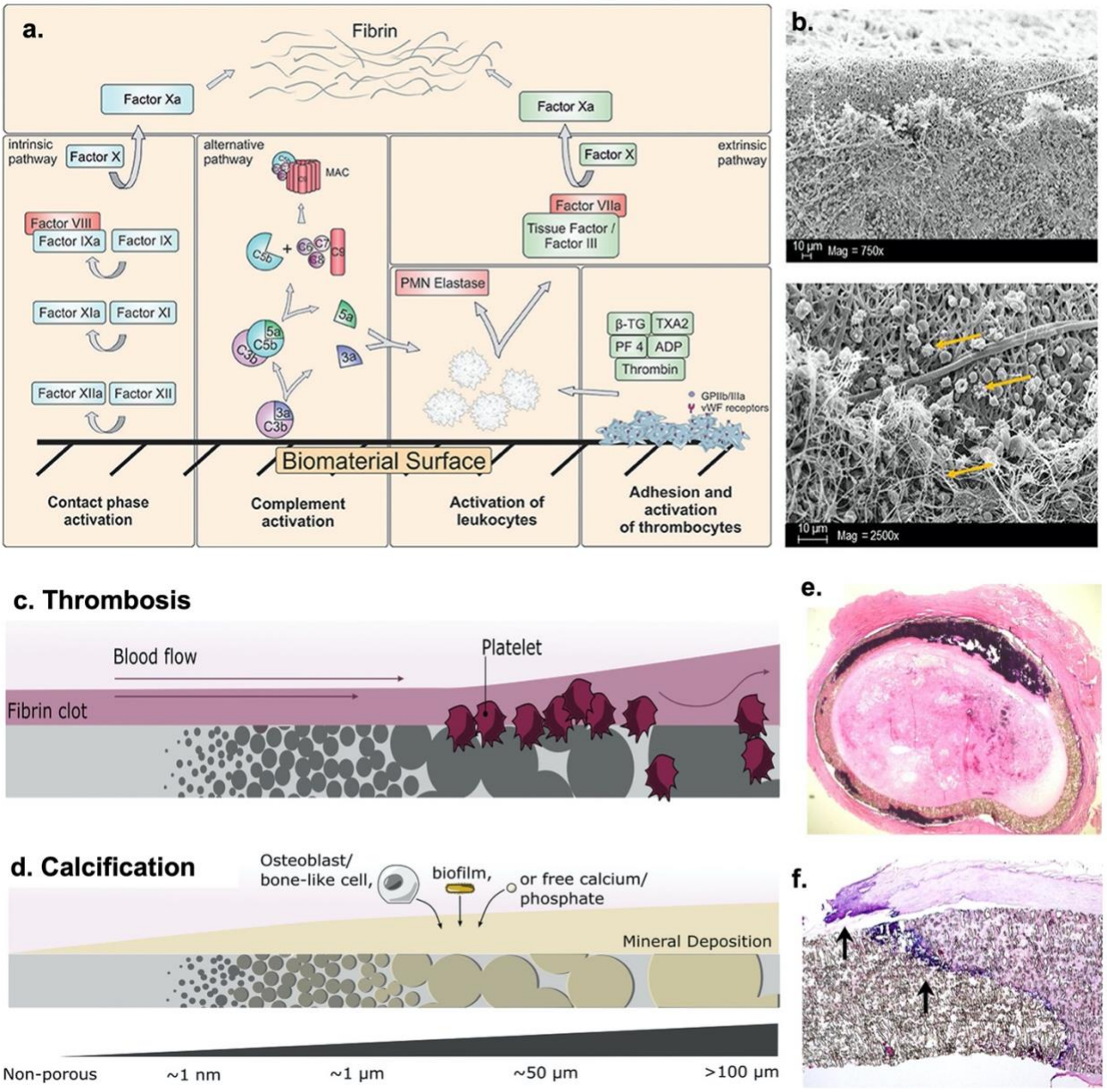

Figure 1. Adverse reactions induced by blood-contacting implants. a) Schematic illustration of the activated intrinsic and extrinsic coagulation pathways that results in a fibrin network. b-c) Biocompatibility trends in respect to biomaterial pore size. b) Thrombogenicity development and shear mediated platelet activation with large porosity and surface roughness. c) Calcification deposition in larger pored materials sourced from osteoblast-like cells, a biofilm, or free minerals.[14] d) Scanning electron microscopic (SEM) analysis of artificial vascular grafts after activation of the blood coagulation system illustrating the adhered platelets and the 3D-fibrin meshes shown by the yellow arrows.[15] e) Thrombus formation on PTFE vascular grafts. f) Transmural calcification within PTFE vascular grafts at the edge of fibrin deposits as shown by the arrows.[16]

2.1. Thrombosis

Nonspecific protein adsorption is the initial event at the interface between the surface of the implant and human blood, wherein the dynamics of protein adsorptions are governed by kinetic and thermodynamic principles. Although the blood contains over 300 proteins of varying shape, size, and concentration, the main contributors to the formation of the non-specific protein adsorbed layer are fibrinogen, albumin, and immunoglobulins.[12] Typically, the first plasma protein to be adsorbed to the surface is fibrinogen following other contact factors, and adsorption of even small amounts of fibrinogen can initiate a cascade of events including platelet adhesion and activation of coagulation (Figure 1d). Locally generated thrombin converts fibrinogen to fibrin and the fibrin monomers then polymerize to form fibrin. Thrombin also serves as a potent platelet agonist, which activates ambient platelets that adhere and aggregate on the graft surface.[17] Formation of platelet-rich thrombi on the graft surface fouls the device and can provide a nidus for infection, or can reduce graft patency and lead to embolic complications (Figure 1e).[18] Complement activation by synthetic vascular grafts could also lead to the prevention of endothelial cell seeding, resulting in the aggregation of platelets and the in situ production of tissue thromboplastin by polymorphonuclear leukocytes. Aside from thrombosis, fibrosis and calcification are other long term complications of synthetic vascular grafts.[19]

2.2. Calcification

Calcification of prosthetic vascular conduits is a frequent complication of inadequate biocompatibility. A study reported that of 40 failed PTFE bypass grafts, 68% revealed calcifications within 1 month after implantation.[16] Characterized by ectopic mineral formation in the form of calcium phosphate or other calcium salts (Figure 1f), calcification can increase the risk of heart attack, stroke, and limb amputation by contributing to vascular occlusion and through embolization of calcific deposits.[19] Additionally, vascular mineralization results in mechanical

dysfunction of implants. Although recent research suggests that calcification is an active cell-mediated process, the details of its pathogenesis remain uncertain. Factors related to this phenomenon include matrix remodeling, endoplasmic reticulum stress, apoptosis, inflammation, and the generation of reactive oxygen species (ROS).[17,19] Because of the high prevalence of interstitial calcification, vascular graft materials that are resistant to calcification are urgently needed.

3. Functional Modification Approaches for Vascular Grafts

Surface characteristics including surface wettability, potential, energy, topography, and reactivity govern the hemo and cell compatibility of artificial grafts (Figure 2). Superhydrophobic surfaces characterized by low water sliding angles (<10°) and high-water contact angles (>150°) reveal reduced protein adsorption and less blood-material interfacial contact. Surface topography including wrinkles, wells, pillars, and/or other structural formations can modulate cell and protein adhesion and attenuate thrombosis by altering the surface area and energy.[20] Similarly, surface charge and chemical reactivity may alter the hemocompatibility by modulating the interaction of surface groups and blood species at the blood-material interface. Chemical structures such as sulfonic groups can reduce protein adsorption and contact activation with blood, whereas carboxyl groups tend to heighten platelet activation and thrombin generation. The surface energy, surface charge, and chemical composition of materials act in concert to determine the likelihood of thrombosis and calcification.[3,9,10] Below is a review of current functional modification strategies that have been applied to blood-contacting materials.

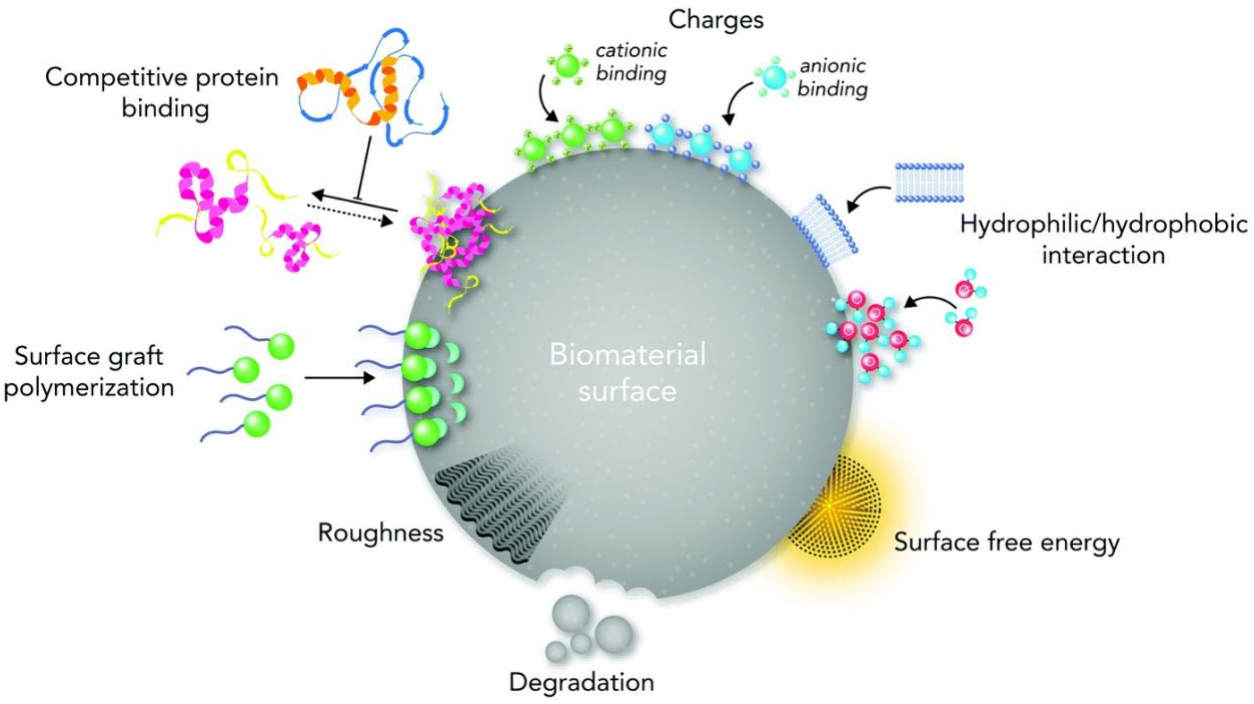

Figure 2. Biological responses of a biomaterial surface based on the key surface physiochemical properties. Manipulation of the molecular and cellular signaling pathways based on topography, stiffness, functional groups, biological moieties, ions, charges, and surface free energy.[10]

3.1. Prevention of calcification

Early studies in the field of surface modification of vascular grafts mainly focused on the prevention of passive deposition of calcium within the lumen of the grafts and around them. Compared with uncoated grafts, vascular prosthetic grafts coated with heparin exhibited significantly reduced calcium content 5 months after subcutaneous implantation in rats.[21] Likewise, coating a portion of a porous polyurethane graft with sulfonated poly(ethylene oxide) reduced platelet adhesion and dissolved calcium in a canine right ventricle-pulmonary artery shunt model compared with the uncoated portion. These findings are thought to reflect the non-adhesive and mobile characteristics of poly(ethylene oxide), as well as the negative charge of sulfonate acid groups.[22] However, both types of coatings were associated with increasing calcium deposition over time, suggesting that the coatings delayed but did not abolish the calcification process.[21,22] This is

due to the fact like bone formation, calcification is an active process that progressives over time and cannot be hindered by passive measures.[19]

3.2. Antithrombogenic

Thrombosis is the primary barrier to the long-term patency of vascular grafts. Strategies adapted for antithrombogenic applications mainly involve coating the surfaces with anticoagulants such as heparin or vasodilators and inhibitors of platelet activation such as nitric oxide, inhibitors of protein adsorption such as poly(ethylene glycol) (PEG) or zwitterionic polymers, and synthetic drugs.[1,10,34,36,23,24,25]

3.2.1. Anticoagulants

Various anticoagulants have been used to improve the hemocompatibility of vascular grafts such as heparin, corn trypsin inhibitor, as well as direct thrombin inhibitors such as hirudin, bivalirudin, and argatroban.[3,10] Among these, heparin is the most widely used agent. Heparin acts as an anticoagulant by binding to antithrombin and accelerating the rate at which it inhibits thrombin, factor Xa and other coagulation proteases (Figure 3a). Various techniques have been used to immobilize heparin on surfaces including covalent attachment, physical adsorption, electrostatic attachment, and layer-by-layer deposition.[3] Covalent attachment of heparin is the preferred method because each heparin molecule possesses several free carboxyl groups that enable covalent bonds with functional groups on the biomaterial such as amino or hydroxyl groups.[10] However, the efficacy of the immobilized heparin can be compromised because the multiple covalent linkages limit its capacity to bind to antithrombin. End-point immobilization of heparin reduces this problem and materials coated with heparin using this technique exhibit less platelet adhesion and clot formation compared with materials coated with heparin via multivalent techniques. Several heparin coated grafts are currently available including Gore® and Gentige®, which are fabricated

from ePTFE and Dacron, respectively.[3] However, the applicability of these grafts is hindered by the short half-life of the immobilized heparin.[1,26] The half-life of heparin may be prolonged by using "sandwiched" layer-by-layer surface coating methods. For example, when an electro-spun polycaprolactone vascular graft was modified with polyethyleneimine and heparin using catechol/gallol surface chemistry, there was more heparin on the surface compared with other conventional layer-by-layer coating methods and the graft's capacity to resist platelet and fibrinogen adhesion were prolonged.[23]

Endogenous anticoagulant agents have been extensively explored for use in anticoagulant coatings. Anticoagulant proteins such as tissue factor pathway inhibitor and protein C exhibit thromboresistance *in vitro*, however their bioactivity was compromised by the sterilization process.[24]

3.2.2. Endothelial Cells (ECs)

Vascular grafts coated with endothelial cells (ECs) have shown great potential in preventing acute thrombosis compared with other agents. As ECs continuously undergo shear stress, they stimulate the release of nitric oxide by endothelial nitric oxide synthase, which triggers vasodilation, inhibits platelet aggregation, reduces restenosis, and enhances human umbilical vein EC adhesion (Figure 3b).[1,27] When a polyurethane vascular grafts coated with a polymer containing a nitric oxide donor was evaluated in a sheep arteriovenous bridge-graft model, there was no platelet adhesion or thrombus formation after 21 days. In contrast, uncoated grafts exhibited adherent thrombi and red blood cell and inflammatory cell infiltration into the graft wall.[25]

3.2.3. Bioinert Polymers

Bioinert polymer coatings resist nonspecific adhesion of proteins and cells by binding water molecules to form a hydration layer that attenuates thrombosis. Among different hydrophilic

polymers such as dextran and tetraethylene glycol dimethyl ether, the most commonly used synthetic polymer is PEG. The repellency properties of PEG are highly dependent on its chain length and surface density.[10] Some studies have reported enhanced protein and platelet adhesion on high-density PEG coated surfaces, whereas others found that increasing the surface grafting density creates a more porous and rougher surface coating that promotes cell adhesion. Despite the several promising antithrombogenic results of PEG coated implants, long-term *in vivo* tests revealed depletion of the coated PEG layer and degradation of its chain length and surface density due to oxidation by ROS. Therefore, PEG-coated surfaces are only useful for short-term applications.[10,28] In contrast, vascular grafts coated with zwitterionic polymers have shown superior antithrombogenic results, due to their innate antifouling structure composed of a phospholipid rich cell membrane containing zwitterion head groups of carboxybetaine and phosphorylcholine. Studies of zwitterion structures for blood contacting applications have commonly used 2-methacryloyloxyethyl phosphorylcholine (MPC) due to its simple, high purity, and high-yield fabrication process, in addition to the high free-water fraction levels of its polymer chains that reduce the protein adsorption force at the interface (Figure 3b).[9,29] A study using a MPC coated poly(ester urethane)urea graft implanted as an aortic interposition grafts in rats for 24 weeks showed a tenfold decrease in platelet adhesion relative to uncoated grafts. Furthermore, immunohistochemical staining revealed formation of smooth muscle and endothelial cell markers on the coated grafts, as well as oriented collagen and elastin deposition. However, further *in vivo* experiments are required to substantiate the applicability of these coated grafts for complete remodelling into a native-like artery.[29]

3.2.4. Synthetic Drugs

Other coatings such as drug eluting vascular grafts have been designed to slowly release bioactive substances into the bloodstream. Synthetic drugs that release reactive-oxygen-induced antiplatelet ethyl salicylate have been shown to prevent blood clotting.[1] More recently, a decellularized tissue engineered vascular graft coated with a hyaluronic acid hydrogel revealed antithrombogenic protection and promotion of endothelium, due to protection of the underlying collagen layer. There were fewer adherent platelets and less fibrinogen and fibrin formation on coated grafts than on uncoated grafts. Furthermore, *in vitro* studies showed inhibition of macrophage adhesion due to the strongly hydrophilic nature of hyaluronic acid. Studies *in vivo* in rat and dog models showed reendothelialization after 5 weeks (Figure 3d). These finding pave the way for an "off-the shelf" tissue engineered vascular graft for small diameter applications.[30]

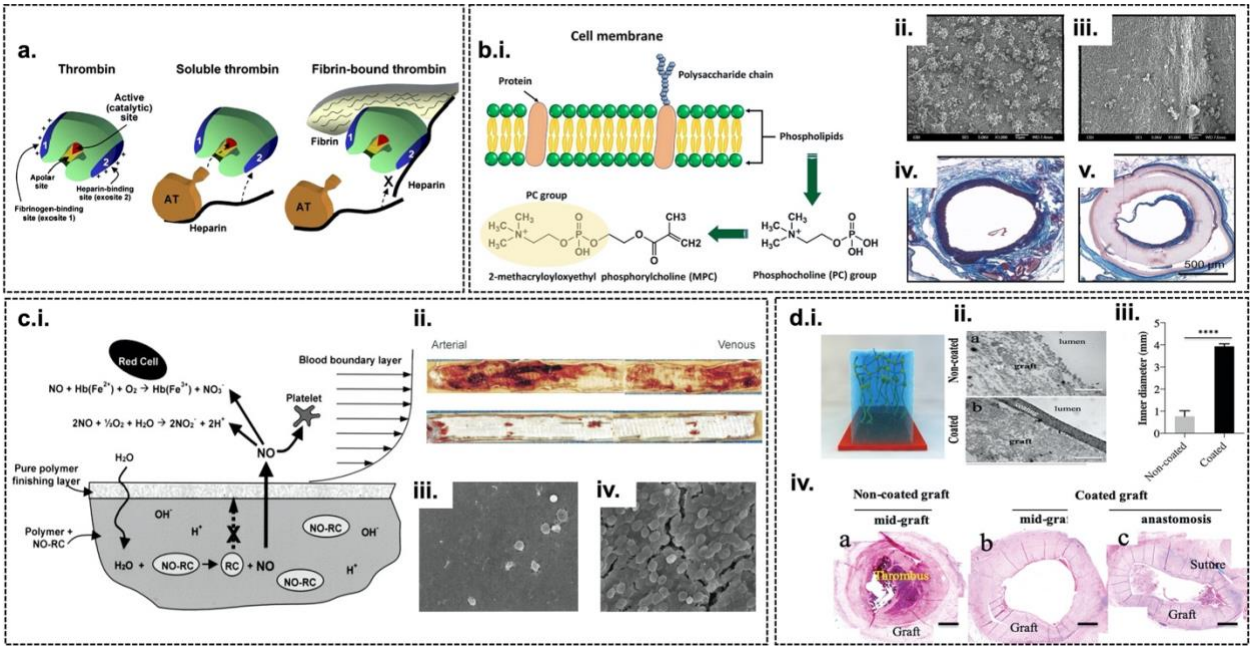

Figure 3. Antithrombogenicity of vascular grafts. a) Schematic illustration of thrombin and its inhibition.[31] b,i-iv) Nitric oxide-releasing polymer coating on prosthetic vascular grafts. i) Schematic illustration of nitric oxide release from polyvinyl chloride biomaterial upon contact with water. ii) Photographs of explanted and longitudinally opened polyurethane vascular grafts displaying luminal thrombus on uncoated (top) and coated (bottom) grafts. iii-iv) SEM images showing platelet adhesion and thrombus accumulation from arterial anastomoses on iii) coated and iv) uncoated grafts (Magnification ×1000).[25] c,i) Design and synthesis of the zwitterionic polymer, MPC, depicting its phospholipid rich cell membrane and phosphocholine functional groups.[10] ii-

iii) SEM images assessing the thrombogenicity after exposure to ovine blood on ii) uncoated vascular graft showing platelet aggregates and iii) MPC coated vascular graft free of platelet depositions. iv-v) Histology assessment post a 24-week period *in vivo* of the MPC coated vascular graft, depicting i) a section of a native rat abdominal aorta prior to the proximal anastomosis of the graft and ii) section taken in proximity of the distal anastomosis.[29] d,i-iv) Hyaluronic acid modified surface coating on decellularized TEVGs vascular graft. i) Depiction of hyaluronic acid coating on vascular graft. ii) Transmission electron microscopy (TEM) images of the coating morphology of uncoated and coated hyaluronic acid coated tissue engineered vascular graft. iii) Comparison of the inner diameter of the uncoated and coated allografts in rat aortic implants at 32 days ($n = 6$, $*p < 0.05$, $**p < 0.01$, by unpaired, two-sided t-test with Welch's correction). iv) Images of hematoxylin & eosin stained clotted, uncoated tissue engineered vascular grafts and coated graft at both the b) mid-graft and c) anastomosis regions of canine explants.[30]

3.3. Endothelialization of Vascular Grafts

The formation of a confluent endothelial layer on newly implanted vascular implants is essential for attenuation of inflammation and coagulation. Recent innovative methods have focused on the *in-situ* engineering of the interface between vascular grafts and the blood to capture circulating ECs with EC specific ligands or cell adhesion molecules, thereby accelerating endothelialization. Biomimetic nanofibrous scaffolds provide an ideal surface to volume ratio and abundant binding ligands to enhance EC adhesion. Alternatively, circulating ECs can be captured with immobilized monoclonal antibodies against CD34. Studies performed *in vitro* with implants coated with anti-CD34 antibody in a functionalized multilayer containing heparin-collagen revealed enhanced viability and metabolic activity relative to non-antibody functionalized surfaces. Additionally, *in vivo* experiments showed less neointimal hyperplasia compared with non-functionalized surfaces.[10] Other studies examined surfaces coated with vascular endothelial growth factor receptor (VEGFR) to capture ECs on hydroxyl terminated poly (ethylene-co-vinyl alcohol) surfaces. VEGFR surface coating significantly increased the number of cells expressing VEGF after 2 weeks in culture and induced their differentiation (Figure 4a).[32] Alternatively, coating grafts with active peptides with simple structures and high stability has been shown to promote EC adhesion. The most employed peptide is Arg–Gly–Asp (RGD), which was identified as the

minimal essential cell adhesion peptide sequence in fibronectin. RGD can be immobilized on the surface of biomaterials by binding to hydroxyl, amino, or carboxyl groups (Figure 4b). Studies have shown that a high RGD density promotes rapid proliferation of ECs, which can be controlled using bioactive motifs such as Lys and glycine molecules at the N-terminus of peptides that act as spacers (Figure 4c). Although promising, RGD peptide also promotes the adhesion of platelets, thus novel peptides need to be screened with selective binding biofunctions to the special integrins in ECs.[11]

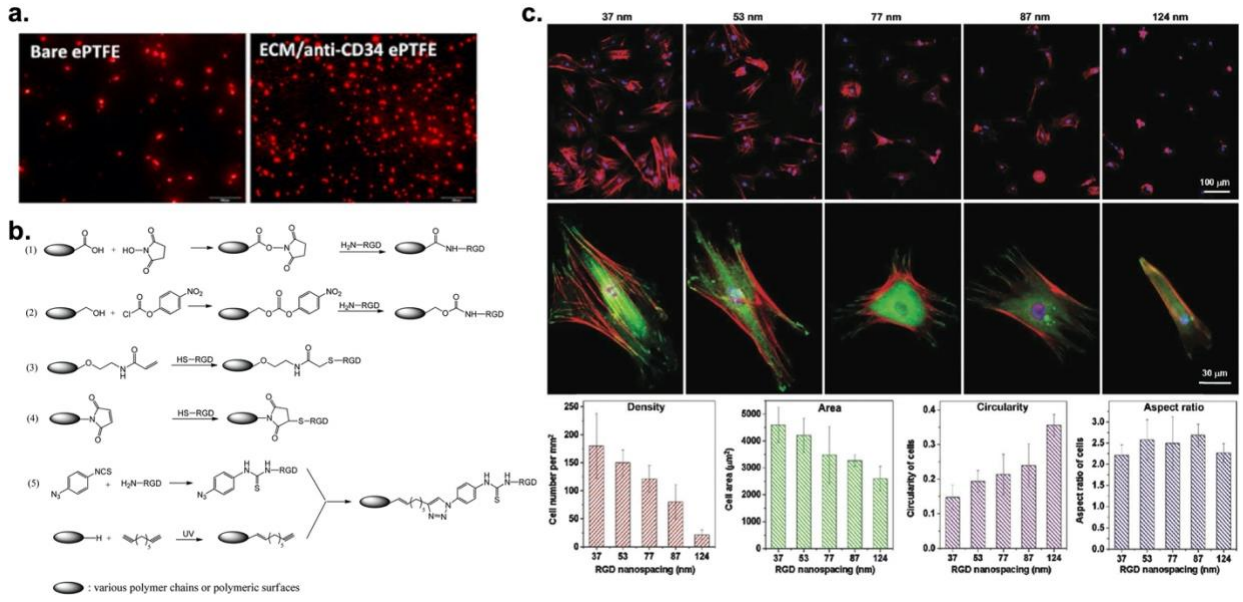

Figure 4. Promotion of endothelization on vascular grafts. a) Investigation of *in vitro* cell adhesion on uncoated and anti-CD34 coated ePTFE grafts after 24 hours of blood perfusion.[10] b) Different immobilization methods of RGD peptide on biomaterial surfaces by activation of the carboxyl acid groups on the material's surface with NHS; activation of the hydroxyl groups on the material's surface with p-nitrophenyl carbonate; thiol-RGD peptide reacts with acrylic derivatives via the Michael addition reaction; thiol-RGD peptide reacts with maleinimide via the Michael addition reaction; immobilization of the RGD peptide via the azido–alkyne click reaction. c) Cell adhesion on nanopatterned surfaces with various nano spacings with cells stained red for F-actins, green for vinculins, and blue for nuclei, wherein the top and middle rows reveal low-magnification and high-magnification fluorescent micrographs of smooth muscle cells cultured on nanopatterns for 24 h. Bottom graphs show the statistical results of the cell adhesion.[11]

3.4. Lubricant-infused Surface (LIS) Coatings

While the above-mentioned strategies have shown promising results, they rely on a single line of defense that renders thrombosis a problem once the coating is depleted. Although bioinert coatings provide a second line of defense, the lack of endothelialization is problematic because they lack bifunctionality. To overcome the limitations of the coatings reported thus far, LIS provides a highly versatile coating consisting of an underlying flat or rough solid substrate that locks onto the surface through van der Waals and capillary forces and provides a thin, stable, and dynamic perfluorinated lubricant layer.[33–40] By either exploiting the innate chemical properties of the substrate or chemically modifying the substrate using silanization techniques (Figure 5a), a compatible-lubricant infused platform is created which energetically favors the lubricant layer rather than other contaminating liquids and forms an interface that repels aqueous, organic, and complex biological fluids, as well as prevents cell adhesion and biofilm formation.[10]

Since Teflon is synthesized from a fluorocarbon-based polymer, it does not require further chemical modification because its porous structure is compatible with perfluorinated lubricants. In contrast, epoxy-resin-based substrates need to be chemically modified through silanization with a fluorosilane monolayer prior to infiltration with the lubricant layer. Silanization techniques mainly involve liquid phase deposition (LPD) or chemical phase deposition (CVD). However, the results of studies with slippery lubricant-infused coronary catheters modified using LPD or CVD techniques suggest that CVD treatment provides better resistance against thrombosis than LPD treatment. This finding mainly reflects the exposure to acids in the liquid phase during LPD treatment, which produces etching and damage to the inner layers.[41–43] Common lubricants reported in the literature include Krytox-100, Krytox-103, perfluoroperhydrophenanthrene, perfluorodecalin, perfluorohexane, and perfluorooctane.[44]

LIS coatings have been extensively used in healthcare applications and have shown compatibility with medical grade materials such as poly(methyl methacrylate), polyethylene terephthalate, ePTFE, polyether amide, polycarbonate, and polyurethane.[3,45] LIS coated implants have been shown to outperform conventional surface coatings by efficiently reducing clot formation and device associated infection. For example, PTFE-based LIS coating infiltrated with Krytox-103 revealed a 35-fold reduction in biofilm formation compared with PEG-coating. Additionally, compared with untreated grafts implanted in rats, lubricant infused ePTFE grafts exhibited a 99% reduction in *Staphylococcus aureus* adhesion and a reduced local inflammatory response.[20,46]

Although LIS coatings have shown exceptional antithrombogenic results, they lack bio-functionality and inhibit all bio-interactions with the surface. To address this issue, bio-functional lubricant-infused (BLIS) surfaces were developed to allow targeted binding of ECs and tissue integration on the interface without compromising the repellency of the surface (Figure 5b). BLIPS were created on oxygen plasma treated ePTFE vascular grafts using (3-aminopropyl) triethoxysilane (APTES) silanized anti-CD34 bio-links, infiltrated with perfluoroperhydrophenanthren lubricant. Relative to uncoated ePTFE grafts, BLIPS attenuated thrombin generation and resisted blood clot formation and non-specific protein adhesion. In addition, a confluent endothelial layer was observed on the coated surfaces after incubation with a blood/EC mixture for four days reflecting targeted binding of ECs from the complex mixture (Figure 5c).[10] The simple fabrication process of BLIPS and their outstanding antithrombogenic performance render these coatings a promising candidate for the next generation of synthetic vascular grafts. Further studies are necessary for the translation of these coatings to real life applications, such as optimization of the bioactivity of immobilized agents, as well as the stability and durability of the coatings for long-term applications.[47,48]

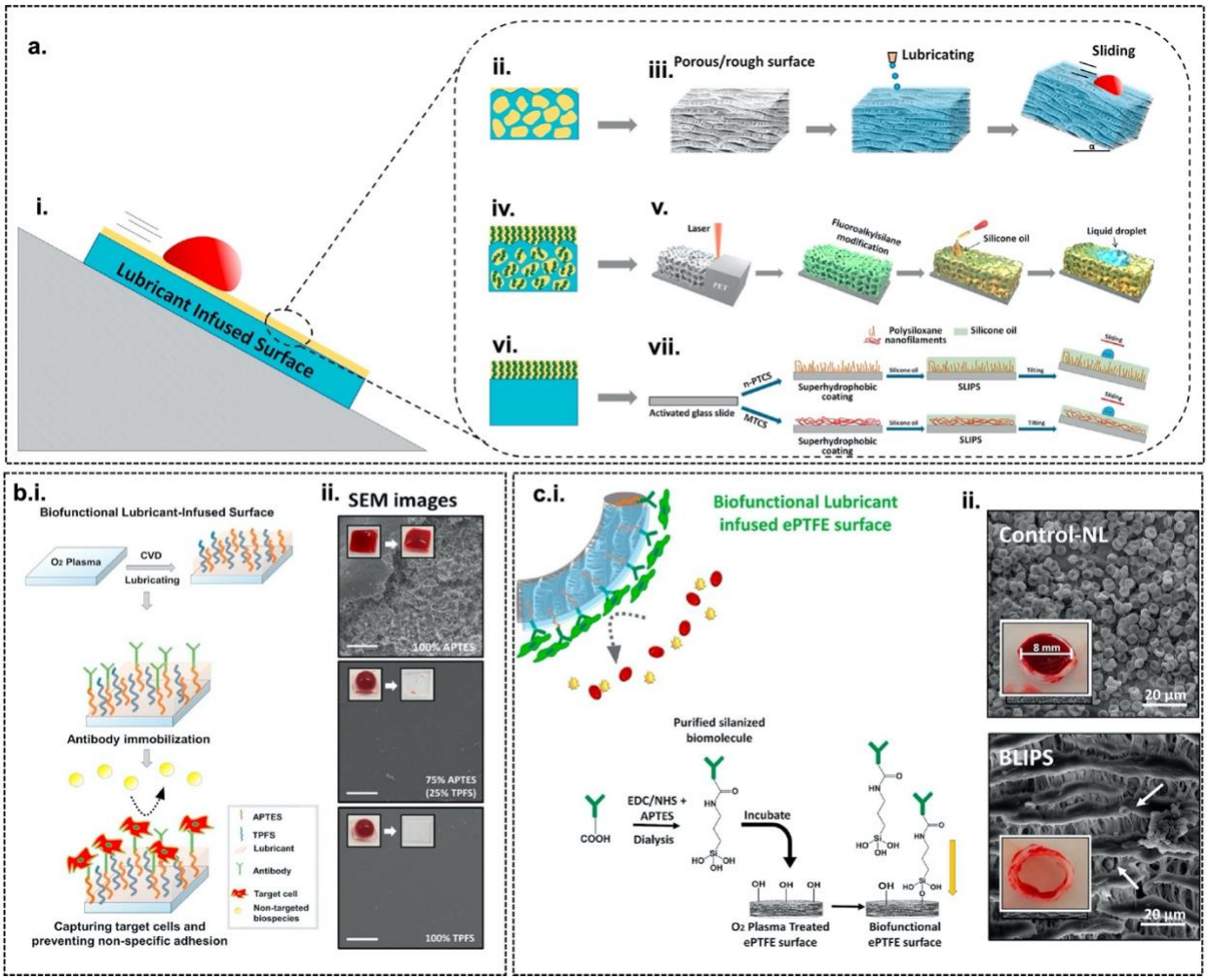

Figure 5. LIS coated vascular grafts. a,i-vii) Illustration of the wetting behaviour of LIS coatings. ii-iii) LIS PTFE/ ePTFE nanofibrous membranes created using innate chemical properties of the rough surface. iv-v) Chemical modification of porous PET surfaces by femtosecond laser direct writing and functionalization with fluoroalkyl-silane followed by inflation with silicone oil. vi-vii) Chemical modification using oxygen plasma treatment on flat glass substrates and functionalization using n-propyltrichlorosilane (n-PTCS) or methyltrichlorosilane (MTCS) and infiltrated with silicone oil to create polysiloxane nanofilament coatings.[10] b,i) Fabrication of BLIS coated with anti-CD34 antibody using CVD of mixes silanes and ii) SEM images depicting the plasma clotting assay experiments.[49] c,i) Development of BLIS on ePTFE grafts using silanized bioinks and ii) SEM images from blood clotting assay depicting the significant reduction of blood clot adhesion on BLIPS relative to non-lubricated control ePTFE grafts.[10]

4. Translation of Antithrombogenic Coatings to Clinical Applications

Prior to implantation, the hemocompatibility of implants needs to be analyzed and must fulfil the guidelines developed by the International Organization for Standardization (ISO 10993-4).[50]

Several *in vitro* testing models are available to assess the hemocompatibility of implants by incubating them in human blood under static, agitated, or shear flow conditions.[13,15]

The first step is evaluation of the thrombogenicity of blood-contacting devices under static conditions. Subsequently, assessment of the hemodynamic behaviour under dynamic flow is crucial, namely the shear-induced activation of blood cells and proteins, as well as the flow dependent transport of procoagulant cells and proteins. Recognizing the importance of the interaction of blood flow and the biomaterial surface in a dynamic context, studies have focused on developing *in vitro* testing platforms such as tubular and cone-and-plate viscometers, parallel plate flow chambers, and microfluidic flow chambers. Among these techniques, the use of microfluidic devices provides the most efficient approach because of the short analysis times, versatility of fabrication protocols, minimal consumption of reagents, high resolution and sensitivity of fluid manipulation, and portability (Figure 6b). The advantages of microfluidic devices have driven the development of organ-on-a-chip (OOC) technology to model conditions present in the human bloodstream, specifically vascular-like flow conditions and vascular-like microenvironments. Recently, a microfluidic platform for *in vitro* testing was fabricated to compare the thrombogenicity of LIS and anti-CD34 coated ePTFE vascular grafts under arterial wall shear stress and with and without ECs under flow conditions. Using this "vascular graft-on-a-chip" device, the thromboresistance of the coated vascular grafts was established under flow conditions and the attenuated thrombin generation and fibrin deposition was monitored in real time, thus facilitating the translation of synthetic grafts into the clinical space (Figure 6c). Once results from benchtop testing have met the guidelines developed by ISO 10993-4 and the biomaterial is safe for use *in vivo*, computational *in silico* testing and studies in animals are the next steps.[3,15] Common animal species used for such testing include mice, rabbits, goats, and pigs.

Computational fluid dynamics and mathematical algorithms are mainly used to model thrombus growth, platelet activation, and platelet aggregation for *in silico* models.[3]

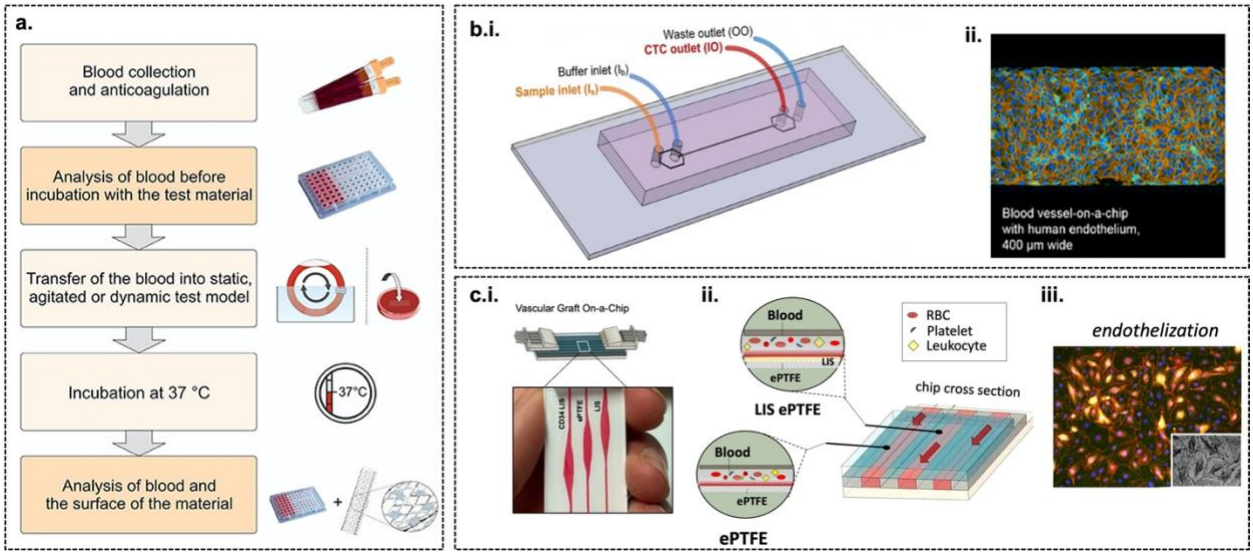

Figure 6. Evaluation of the hemocompatibility of synthetic grafts. a) Representation of the procedure to analyze the interaction of blood cells and proteins with a biomaterial surface.[15] b) Testing the hemocompatibility of a vascular graft ii) using a microfluidic chip with an ii) image depicting a fluorescently labeled endothelium in the channel. c,i-iii) Schematic of a vascular graft-on-a-chip to test the thrombogenic performance of LIS and anti-CD34 coated ePTFE vascular grafts in the presence of arterial wall shear stress with and without endothelial cells.[3]

5. Conclusion and Future Directions

Despite the progress in the fabrication and modification of synthetic vascular grafts over the years, many of these coatings have only been tested *in vitro* and if evaluated in animal models, have only shown promise for short-term applications. Novel strategies that exploit the regenerative biological pathways may lead to new synthetic vascular materials or/and coatings that permit vascularized, non-fibrotic tissue reconstruction. LIS coatings hold great promise as a surface modification strategy for vascular grafts, although further research on the stability and durability of the lubricant layer is necessary.

The development of stable, biocompatible, and efficient surface coatings is essential for long-tern applications. The fabrication of biocompatible synthetic vascular grafts that promote

endothelization will pave the way for more advanced treatment options and customizable products for the various clinical applications and pathological environments.

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