Published in final edited form as:

Ann Biomed Eng. 2019 February ; 47(2): 366–380. doi:10.1007/s10439-018-02171-3.

A review of integrin-mediated endothelial cell phenotype in the design of cardiovascular devices

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Abstract

Sustained biomaterial thromboresistance has long been a goal and challenge in blood-contacting device design. Endothelialization is one of the most successful strategies to achieve long-term thromboresistance of blood-contacting devices, with the endothelial cell layer providing dynamic hemostatic regulation. It is well established that endothelial cell behavior is influenced by interactions with the underlying extracellular matrix (ECM). Numerous researchers have sought to exploit these interactions to generate improved blood-contacting devices by investigating the expression of hemostatic regulators in endothelial cells on various ECM coatings. The ability to select substrates that promote endothelial cell-mediated thromboresistance is crucial to advancing material design strategies to improve cardiovascular device outcomes. This review provides an overview of endothelial cell regulation of hemostasis, the major components found within the cardiovascular basal lamina, and the interactions of endothelial cells with prominent ECM components of the basement membrane. A summary of ECM-mimetic strategies used in cardiovascular devices is provided with a focus on the effects of key adhesion modalities on endothelial cell regulators of hemostasis.

Keywords

Coagulation; integrin; cardiovascular devices; endothelial cells; hemostatic regulation

1. Introduction

A critical limitation of early blood-contacting medical devices was their propensity to fail due to thrombus formation from poor biomaterial hemocompatibility. Aggregation of activated platelets can occlude blood vessels and lead to downstream morbidity due to emboli that travel to the patient's lungs or brain. These early failures led to a critical investigation of methods to prevent coagulation, namely the generation of anti-thrombotic coatings and hemocompatible biomaterials for medical devices. Current research aims to understand and recapitulate the body's anti-thrombotic surfaces to improve the patency of blood-contacting medical devices. The endothelium provides dynamic hemostatic regulation

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of all blood-contacting surfaces in the body. Thus, promoting endothelialization of cardiovascular devices is a popular strategy for generating long-term thromboresistance and controlling coagulation without the need for systemic anti-platelet therapies. Endothelial cells prevent platelet activation, provide a protective and selective barrier to underlying tissues, respond to injury, and activate clotting when necessary.[110, 123, 129, 155] Importantly, the endothelial cell layer is a dynamic system that accomplishes all of these tasks by responding to cues not only from circulating blood but also from the underlying extracellular matrix (ECM).[138] These cues can induce changes in endothelial cell phenotype with a resulting change in hemostatic regulation through the expression and release of anti- or pro-thrombotic constituents.[4, 5, 13, 47, 97, 104, 108, 118, 122, 127, 132, 141, 153, 154] Although initial efforts were focused primarily on creating substrates that support endothelial cell adhesion and migration, it is also important to understand the influence of these substrates on the endothelial cell phenotype in regards to hemostatic regulation. The ability to select particular interactions between the endothelium and substrates that limit platelet aggregation and thrombosis will enable advancements in thromboresistant biomaterial design.

The compositional make up and material properties of the ECM vary throughout the body to provide the appropriate cell-material interactions depending on the function of the local tissue. The basement membrane is the ECM that supports endothelial cells in the cardiovascular system with major components consisting of collagen, laminin, nidogen, proteoglycans, and glycosaminoglycans.[72, 73, 156] Endothelial cells bind uniquely to these components using different transmembrane proteins, such as integrins and syndecans,.[72, 74, 75] Integrin and syndecan binding to ligands on the basement membrane initiates intracellular signaling cascades that affect many cell behaviors including migration, proliferation, apoptosis, and hemostatic regulation.[62, 74, 89, 101, 103, 109–111] However, the limited understanding of the individual effects of these relationships has limited thromboresistant biomaterial design. Elucidating key relationships between integrin binding, signaling cascades, and the corollary changes in a cellular hemostatic regulators has become an area of interest for researchers. Elucidation of the key mediators of anti-thrombotic cell behavior can provide improved material design of thromboresistant coatings for blood-contacting devices.

This review will first provide an overview of endothelial cell regulation of hemostasis as it relates to preventing platelet activation and coagulation. A summary of the individual components of the basement membrane and basal lamina and the modalities used by cells to attach to the ECM will then be identified as key regulators of coagulation. Finally, ECM-mimetic strategies used in cardiovascular devices will be reviewed with a focus on the effects of these adhesion modalities (e.g. integrin, syndecan attachment) on endothelial cell behavior.

2. Endothelial Cell Regulation of Hemostasis

Endothelial cells have several mechanisms for regulating coagulation and inflammation.[147, 167] In addition to providing a physical barrier to the pro-thrombotic ECM, endothelial cells are responsible for the initiation or direct regulation of coagulation, platelet function, and fibrinolysis to minimize adverse consequences of vascular injury, as well as maximize

vascular repair capabilities.[119, 123, 145] Disruption of these regulatory functions can lead to cardiovascular disease and eventually death, highlighting the importance of the endothelial layer in cardiovascular systems.[2, 11, 26, 36, 39, 95, 110, 123, 155] For example, in Marfan's Syndrome, mutations in fibrillin-1 result in endothelial dysregulation characterized by low nitric oxide (NO) production that leads to numerous complications in the disease state.[24] Therefore, in order to understand the effects of various influencers on endothelial cell hemostatic regulation, it is important to describe this functionality in full.

In controlling coagulation, the endothelial cells bind antithrombin III that is responsible for inactivation of thrombin, Factor Xa, and Factor IXa in the coagulation pathway, slowing coagulation.[123] The ECs also express thrombomodulin, which in turn promotes the activation of protein C in concert with endothelial protein C receptor.[43, 56] Activated protein C is an anticoagulant that limits the conversion of Factor VIII to Factor VIIIa and prevents the conversion of Factor V to Factor Va.[43, 56] Endothelial cells also prevent coagulation by expressing tissue factor pathway inhibitor (TFPI) which inhibits the conversion of Factor VII to Factor VIIa.[162] Without these controls, the coagulation pathway would proceed unchecked and clotting would be prolific in the body leading to increased rates of stroke, embolisms, and heart attacks.[110, 123]

Platelets play a critical role in coagulation with fibrin-stabilized platelet aggregates able to rapidly form hemostatic plugs upon vessel damage.[120] Endothelial cells can rapidly promote platelet adhesion and activation by producing and releasing von Willebrand factor (vWF), a blood glycoprotein that binds to Factor VIII as a stabilizing agent against protein C, platelet surface glycoproteins, and constituents of the ECM. vWF exists in two compartments in endothelial cells: constitutively secreted pathway where dimerized vWF is exported to the plasma and subendothelial matrix, and residing in a granular store containing very highly multimerized vWF that can be mobilized rapidly in response to agonists such as thrombin.[34] Endothelial cells also produce ADAMTS13, which cleaves the ultra-long vWF strings (ULVWF) that form to capture platelets.[15, 45, 141, 151, 170] Endothelial cells also reduce platelet activation by producing prostaglandin I2 (PGI_2) and endothelial nitric oxide synthase (eNOS).[161] Synthesis of these molecules is triggered by increases of intracellular calcium ion concentrations in endothelial cells.[163] PGI_2 and nitric oxide (NO) are both potent vasodilators and inhibit platelet activation.[78] The powerful anti-aggregatory and vasodilator properties of PGI2 and nitric oxide make them critical regulators of coagulation.

Endothelial cells also play an important role in fibrinolysis, or the enzymatic breakdown of blood clots, an important consideration for medical devices by enabling endothelial cells to help eliminate small clots and prevent large thrombi formation. To this end, endothelial cells synthesize and acutely release tissue plasminogen activator (t-PA), a protein that is involved in the dissolution of blood clots by converting plasminogen to active plasmin. tPA is constitutively released from small granular stores that are separate from vWF stores.[12] Endothelial cells release plasminogen activator inhibitor PAI-1 (the main t-PA inhibitor) in activated conditions to prevent excessive fibrinolysis by blocking the action of t-PA.[12] The regulation of t-PA and PAI-1 is vital to healthy vasculature because an imbalance of either of these factors leads to hemorrhagic disease or hypercoagulable states.

Endothelial cells are responsible for releasing a number of products that trigger signaling cascades that carefully balance the cell response to maintain hemostasis. A summary of the prothrombotic and antithrombotic agents regulated by endothelial cells is provided in Table 1. When disease states are induced in endothelial cells, either from extracellular or intracellular cues, this balance is perturbed. Therefore, it is critical to maintain endothelial cell health when attempting to recapitulate the endothelial cell environment for cardiovascular applications.

3. The Cardiovascular Basement Membrane and Basal Lamina

Most vasculature consists of a tri-layer structure, with the tunica adventitia as the outer layer, the tunica media as the middle layer, and the tunica intima as the inner-most layer (Figure 1). Each layer is separated by a fibrous elastin layer. The intimal layer is of particular interest, as it consists of the basement membrane as the innermost layer and the basal lamina supporting the basement membrane directly beneath it. The basement membrane is crucial for the maintenance of a confluent and functional endothelial cell monolayer that provides the dynamic hemostatic regulation described above.[26, 72, 75] It is typically 20–120 nm thick and can prevent the movement of cells from one layer to the next while selectively filtering molecules that are transported across it.[14, 110] The basement membrane and the basal lamina are composed of many components that work synergistically together to not only promote cell adhesion but also influence cell phenotype and genotype.[116, 126, 174] The basement membrane is composed of collagen, laminin, nidogen, glycosaminoglycans, and proteoglycans.[65, 106, 171] The basal lamina directly beneath it is composed of collagen, fibronectin, laminin, glycosaminoglycans, and proteoglycans in varying concentrations depending on the location of the tissue in the cardiovascular system.[73, 155, 156] Varying these components can change the cell response based on ligand type and availability.[68] Although it is the combined presentation of the individual components that drives cellular functions, understanding how each component contributes to the mechanical and biochemical characteristics of the basement membrane will allow for improved constituent selection when creating substrates for endothelial cell growth, whether for antithrombotic coatings or for investigating cell behavior. There are well described differences between endothelial cells of arteries, veins, and capillaries, and researchers have suggested these differences could be associated with basement membrane compositional differences.[23, 90] However, these differences have yet to be described. There is strong evidence, particularly in the recent comparisons of organ specific venous and arterial endothelial cells, that the basement membrane composition may differ and affect endothelial cell survival and phenotype.[91] There is further evidence that atherosclerotic plaques may be limited by culturing endothelial cells on collagen as compared to fibronectin and fibrinogen.[117] This highlights an important future direction of research that could enhance development of vascular coatings and cardiovascular tissue engineering. Until these differences in basement membrane composition are fully understood, we must rely on the current understanding of individual components to guide cell behavior. A summary of the key components of the basal lamina is provided in Table 2 and detailed in the sections below.

3.1 Collagen

Collagen is the most prominent constituent of the basement membrane and basal lamina and is responsible for tensile strength and cellular adhesion. Although 28 types of collagen have been identified, collagen type I and IV are the most prevalent types found in the cardiovascular system.[128] Other non-fibrillar collagens, such as type VIII (formerly known as endothelial collagen), are also important in ECM signaling to endothelial cells.[79] Collagens have a hallmark triple helical structure and are typically involved in forming fibrillar networks in the ECM that impart strength and structure to the basement membrane.[73] Collagen's rope-like structure provides resistance to tensile forces by carrying stress.[64] Furthermore, collagen is one of the main ECM components responsible for imparting cellular adhesive properties through several receptors including binding sites for the α1β1, α2β1, α10β1, and α11β1 integrins, known as the collagen receptor subfamily of integrins.[67] Attachment to these integrins is promoted via the GFOGER peptide sequence on collagen.[85, 128, 168] In addition to binding via integrins, cells also bind to collagen through syndecan-1.[159] Therefore, collagen is vital to basal lamina due to providing significant mechanical strength and biochemical cues that enable cell adhesion and migration.

3.2 Laminin

Laminin is a key organizer of the basement membrane and basal lamina's structure as it can self-assemble into sheets, bringing together the other ECM components through crosslinking.[172] Laminin is composed of three long polypeptide chains (an α, a β, and a γ chain) held together by disulfide bonds.[149] 18 laminin trimers have been investigated and described, with laminin-1 as the most prominent in the basal lamina. In addition to being an important ECM crosslinker, laminin enables ECM interactions with many different cell types through its diverse binding sites for cellular surface receptors. For example, laminin has binding sites for integrins α3β1, α6β1, α7β1, and α6β4 as well as binding sites for syndecans 1, 2, and 4, creating a diverse array of cellular responses and interactions.[21, 28, 30, 40, 152, 164]

3.3 Fibronectin

Fibronectin, a glycoprotein, is another major constituent of the basal lamina and is formed by two nearly identical polypeptide chains attached via disulfide bonds to form a dimer structure.[42, 121, 137] Fibronectin exists in both soluble and insoluble forms.[12159] Soluble fibronectin circulates in the blood and other body fluids, and insoluble fibronectin is found within the ECM.[144] Although transcribed from a single gene, fibronectin within the ECM has multiple forms as a result of alternative splicing that can generate up to 20 variants.[121] These fibronectin variants promote specific cellular and ECM interactions by generating different adhesive ligands. For instance, fibronectin facilitates cellular attachment of endothelial cells to the ECM via integrin binding sites, primarily integrin α5β1, but also αvβ3, α4β1, α4β7, and α9β1 with RGD, PHSRN, LDV, and REDV binding sites.[58, 92, 121, 125, 176] Additionally, fibronectin not only binds to cells but also promotes adhesion to other ECM components such as collagen, primarily in denatured regions of collagen triple helices through functional and structural domains, as well as heparin and fibrin through

specific binding domains.[121] Overall, fibronectin offers complex interactions of the basal lamina with ECs and their environment.

3.4 Nidogen

Nidogen is a small glycoprotein that makes up about 2–3% of the basement membrane.[148] Nodogen plays a critical role in ECM organization in the basement membrane as it is responsible for cross-linking collagen, laminin, perlecan, fibrinogen, and fibronectin.[1, 25, 41] There are two types, nidogen1 and nidogen2, that are very similar structurally, but have varying relative abundances in different basement membranes.[83] Nidogen2 appears to be the most prevalent type in the vascular basement membrane.

3.5 Glycosaminoglycans and Proteoglycans

Glycosaminoglycans (GAGs) are unbranched polysaccharide chains composed of repeating disaccharide units and are essential to the formation of the ECM as hydrophilic space-fillers in both the basement membrane and the basal lamina. GAGs form gels at low concentrations and allow the ECM to resist compressive forces by hydrating and filling most of the extracellular space.[18, 52, 64] GAGs are considered the “most anionic molecules produced by animal cells” and hydrate the ECM by attracting water molecules due to their high negative charge.[64] GAG chains can be covalently linked to a core protein, forming a proteoglycan.[63] Proteoglycans are abundant in the ECM and can regulate the activities of secreted ECM proteins by binding to them. They play a major role in chemical signaling between cells by changing conformations or blocking of binding sites.[61, 64] Although many proteoglycans are secreted, some remain as trans-membrane proteins, known as syndecans, and act as receptors for ECM proteins.[32] The diverse family of GAGs and proteoglycans are responsible for not only hydration but also ECM-endothelial cell interactions that regulate cellular behavior.

3.6 Elastin

Although it is not a component of the intimal layer that directly affects endothelial cell behavior via binding events, elastin is another major component of other layers of vasculature and provides elastic recovery after stretch or deformation of the tissue that can affect endothelial cell behavior via mechanical cues. Elastin is made from the soluble precursor molecule tropoelastin that generate a highly insoluble crosslinked network.[64, 133, 146] The lysine amino acids of tropoelastin are extensively crosslinked immediately after release from the cell by oxidative deamination of the lysine side chains via the enzyme lysil oxidase with subsequent condensation linking two, three, or four side chains.[29, 64] The elasticity of the resulting elastin network is attributed to the loose, random coil conformation of the resulting polypeptide chain. The stretching of elastin is limited by interwoven stiff collagen fibers, and the overall stress response of ECM is dictated by the interplay and concentrations of collagen to elastin. Elastin has not shown cell adhesive properties and the function appears to be limited to providing important mechanical recoil to tissues.[64]

In summary, the individual components of the ECM work in concert to define the biochemical and mechanical landscape of the basement membrane and basal lamina. Interactions of endothelial cells with these individual components then initiate signaling

cascades to affect cytoskeletal organization and gene expression. Understanding the individual qualities and combined synergistic effects of the basement membrane and basal lamina constituents allows for tailoring of substrates for desired cell growth and behavior.

4 Endothelial Cell Interactions with the Extracellular Matrix

Integrins and syndecans are transmembrane receptors that facilitate ECM-endothelial cell adhesion. These transmembrane proteins uniquely interact with ECM ligands and provide a method for signal transduction from the exterior of the cell to the interior. Understanding which integrins and syndecans are responsible for attachment to individual ECM components in the basal lamina will elucidate the signaling cascades leading to changes in endothelial cell gene expression, Figure 2.

4.1 Integrin Expression in Endothelial Cells

Integrins are critical for not only anchoring cells to the ECM and mediating migration but also important transducers of intracellular signaling that influence cell phenotype.[14051] Integrins are a large family of transmembrane proteins that exist as heterodimers, with 18 unique α and β subunits that combine to form 24 distinct dimers that bind to specific amino acid sequences within ECM proteins.[74] A list of common integrins and their respective ligands are listed in Table 3. Endothelial cells express α1β1 that binds to collagen, α2β1 that binds to collagen and laminin, α3β1, α6β1, and α6β4 that bind to laminin, α4β1 and α5β1 that find to fibronectin, and αvβ3 and αvβ5 that selectively bind to vitronectin.[87, 103, 142] The extracellular portion of these transmembrane proteins links to ligands on the ECM, and the intracellular part of integrins associate with actin binding proteins, including vinculin, α-actinin, paxillin, talin, zyxin, tensin, and filamin.[93] Signaling pathways are then activated by the actin binding proteins which may lead to downstream changes in the chemical or mechanical composition of the ECM, or affect cell behavior such as proliferation, migration, and differentiation.[77, 113] One of the key regulators of integrin-matrix signaling includes the focal adhesion kinase, or FAK, and can play a role in inflammation and hemostasis.[98] Focal adhesion complexes also recruit intracellular proteins such as focal adhesion kinase (FAK), a cytoplasmic tyrosine kinase that plays an important role in cell survival by activating essential signaling pathways critical for the prevention of apoptosis.[27, 69, 74,130, 135] For example, one of the major signaling pathways initiated by the Src-FAK complex is the Ras-MEK-MAPK pathway that affects the transcription of genes important to cell cycle progression.[93]

Not only does the type of integrin affect cell adhesion and signaling, but the location of the integrins and their abundance affect the strength of cellular adhesions and response of the cell. For example, focal adhesion complexes are strong, stable adhesions formed when integrins are clustered on the cell surface, allowing for many cytoskeletal filaments to attach at the resulting plaque.[48] Endothelial cells are dependent on these attachments for cell survival, as detachment from their substrate leads to upregulation of apoptotic signaling.

There are a multitude of other focal adhesion proteins involved in establishing and maintaining cytoskeletal linkages: integrin-bound proteins that directly bind actin, such as talin, α-actinin, and filamin; integrin-bound proteins that indirectly associate with and

regulate the cytoskeleton such as kindling, integrin-linked kinase (ILK), paxillin, and FAK; non-integrin-bound actin-binding proteins, such as vinculin; and adaptor and signaling molecules that regulate the interactions of the proteins of the afore-mentioned groups.[93] These molecules then go on to affect many common cellular pathways such as the Akt, ERK, JNK, RhoA, Rac1, and Cdc42 pathways. Each of these pathways then uniquely affects cell survival, proliferation, differentiation, migration, adhesion, and polarity by modulating gene expression, cell cycle regulation, focal adhesion turnover, and actin dynamics. A review by Legate et al. provides detailed information about the individual pathways and their effects.[93] For example, β1 integrins are involved in a signaling pathway with RACK that results in increased cell migration toward insulin-like growth factor 1, or IGF-1. Binding of the αvβ3 integrin has been shown to induce a β3-SHP-2 interaction that sequesters phosphatase and prolongs IGF1R signaling, that plays an important role in growth and development.[93, 101, 173, 177]

Overall, integrins provide complex and wide-ranging modes of attachment and signaling in endothelial cells. The endothelial cell presentation and available ECM binding sites dictate the resulting cell behavior. However, it remains challenging to isolate the role of one integrin from another in order to discern the individual and synergistic contributions of these interactions. Elucidation of these roles would provide greater understanding of how cells regulate hemostasis and how specific binding events can be incorporated into material design to facilitate that process.

4.2 Syndecans

The evolving roles of syndecans, type I membrane glycoproteins composed of GAG chains covalently linked to a core protein, is becoming increasingly important in understanding ECM -endothelial cell interactions.[10] Syndecans often act as co-receptors to ligands such as vascular endothelial growth factor (VEGF) and fibronectin that have interactions of particular interest in the ECM and are relevant for tissue engineering.[6] The syndecan family is organized into four group members: syndecans-1, −2, −3, and −4, each with distinct functions as shown in Table 4. For example, syndecan-1 regulates cell-interstitial collagen adhesion and binds to fibronectin, as well as growth factors via heparin sulfate chains.[32] Syndecan-1 is down regulated in endothelial cells and can promote differentiation in vascular smooth muscle cells.[22] Syndecan-2 is also found in endothelial cells and binds to ECM components such as fibronectin and growth factors.[32, 44, 166] However, further research is required to understand the specific roles of syndecan-2 in cell adhesion. Syndecan-3 is predominantly found in muscle cells and neuroblastoma cells within the nervous system and binds to certain growth factors.[166] However, it has low affinity for fibronectin, collagen I, III, and laminin and therefore plays a limited role in cell adhesion to ECM.[46, 84] Therefore, syndecan-3 is of limited interest in hemostatic regulation. Syndecan-4 is found to be ubiquitously expressed in all cell types, making it a more widespread component than the other syndecans and is known to be involved in focal cell adhesion, a tight interaction between the cell and ECM, ensuring intracellular signaling.[111] Syndecans influence cell interactions significantly in their roles in cell adhesion and binding to ligands in the ECM.[10] Although the importance of syndecans is established, there is still much to learn about the nuances of their function.[3, 32, 111, 159, 166]

5 Influence of ECM on Endothelial Cell Hemostasis

In an effort to recapitulate the basal lamina, many ECM-mimetic platforms have been used to culture and examine the behavior of endothelial cells.[17, 19] As discussed earlier, endothelial cells bind to ECM proteins via integrins and syndecans, which then initiates intracellular signaling that modulates cell behavior. Although many of the intricacies of the cell-ECM binding are not explicitly discussed in many of the reviewed studies, comparisons of behavior between various ECM components can be used to identify roles of different integrin or syndecan binding and corollary effects on coagulation.

5.1 Collagen-initiated Hemostatic Regulation

Collagen is one of the most prevalent adhesive proteins found in the body and is a commonly selected protein to promote enhanced cellular adhesion to biomaterials. Collagen studies are typically performed using mammalian-derived collagen type I or IV either as a coating or as a crosslinked gel.[49, 53, 94, 96, 165] Endothelial cell adhesion to collagen is mediated by integrins α1β1, α2β1, α10β1, and α11β1 as well as syndecans 1 and 4.[82, 124, 142, 143, 152] These integrins can bind to collagen peptide sequences GFOGER, GLOGERGRO, and GFOGERGVQ.[85, 168] Studies have also been performed on collagen mimics such as these peptide sequences that have more tailored integrin interactions.[49, 112, 139] These typically require coating studies or chemical crosslinking into another network, as they do not form networks on their own.[107]

Collagen coat studies have demonstrated a difference in not only cell behaviors such as migration and proliferation as compared to TCPS but also differences in hemostatic regulation.[53, 96, 112, 165] For example, endothelial cells cultured on collagen-coated ePTFE demonstrated lower levels of PGI_2 and tPA as compared to endothelial cells on an un-coated ePTFE control.[100] This would indicated a less thromboresistant phenotype, but this study did not analyze the production of complementary pro-thrombotic factors in the endothelial cells cultured on these substrates.[100] Another collagen coat study noted an increase in NO production with endothelial cells on the collagen coat as compared to endothelial cells on TCPS, indicating a more thromboresistant phenotype.[49] However, like the previous study, the authors did not discuss the production of pro-thrombotic factors and how the substrate difference would affect their expression. Studies on collagen gels have shown that endothelial cells have decreased expression of PGI2 and vWF, indicating the signaling from collagen attachment is important for endothelial cell regulation of hemostasis.[165]

Studies on collagen mimics that allow for more specific integrin binding are utilized to further elucidate the interactions between endothelial cells and collagen. Streptococcal collagen-like protein, or Scl proteins, are a triple helical protein that are recombinantly expressed in E. coli and have no natural adhesion sites. These proteins have tunable bioactivity as peptide sequences can be inserted via site directed mutagenesis in order to target specific binding proteins.[169] For example, these studies have incorporated the GFPGER sequence targeting integrins α1β1 and α2β1 that are expressed by endothelial cells to bind to collagen and laminin, creating the Scl2 proteins. Endothelial cells showed increased adhesion on PEG hydrogels containing the Scl2 proteins compared to PEG gels alone, as well as comparable adhesion to PEG hydrogels containing collagen.[16, 31]

Endothelial cells seeded on these scaffolds demonstrated a decrease in NOS3 and TM gene expression, and e-selectin gene expression increased compared to collagen gels. This suggests that integrins α1β1 and α2β1 binding are responsible for these gene expression changes.[112]

Based on the evidence in the studies described above and shown in Table 5, the binding of these integrins and syndecans to collagen are responsible for the observed changes in the endothelial cell expression of hemostatic regulators. These integrins could be used as targets to increase the thromboresistance such as through increased NO production. However, parsing out the specific integrin interactions is needed to identify specific integrin binding targets for tissue engineering and cardiovascular device coatings.

5.2 Fibronectin and Gelatin-initiated Hemostatic Regulation

Endothelial cells bind to fibronectin via integrin α5β1, but also α4β1, α4β7, and α9β1 as well as syndecan 4.[58, 92, 121, 176] When endothelial cells are seeded on fibronectin coats, a measured increase in PGI2 as well as tPA was observed as compared to uncoated ePTFE and TCPS.[53, 96, 100] Endothelial cells bind to gelatin also through integrin α5β1 as well as αvβ3, binding specifically to the RGD binding sequence that becomes accessible on denaturation of the collagen triple helix.[35, 57] When gelatin is coated on ePTFE, there are increased levels of PGI2 (anti-coagulant), PAI-1 (pro-coagulant), and tPa (anti-coagulant) compared to the unmodified ePTFE, suggesting a more thromboresistant phenotype.[175] Studies are commonly performed using RGD, as it is a readily available peptide sequence. When endothelial cells are cultured on RGD that is incorporated into hydrogels, ADAMTS-13 (anti-coagulant), TFPI (anti-coagulant), tPA (anti-coagulant), vWF (pro-coagulant), TF (pro-coagulant), P-selectin (pro-coagulant) all increased compared to TCPS.[6] With these increases and results summarized in Table 6, endothelial cells appear to be much more activated on RGD peptides that attach to the integrins α5β1 and αvβ3. Again, these changes highlight specific integrin and syndecan targeting are influencing the endothelial cell's ability to promote coagulation.

5.3 Laminin-initiated Hemostatic Regulation

Endothelial cells bind to laminin via integrins α3β1, α6β1, α7β1, and α6β4 as well as syndecan 2.[21, 28, 30, 142, 164] These integrins commonly bind to laminin peptide sequences that contain YIGSR.[55] Studies on laminin coats have demonstrated an increase in PGI2 expression, indicating a more thromboresistant phenotype of the endothelial cells.[7] Studies have also been performed on laminin-mimetic hydrogels. A laminin peptide sequence targeting syndecan binding was covalently linked to the surface of poly(ethylene glycol) diacrylate hydrogels. Cells grown on these constructs were then compared to hydrogels with RGD binding sequences linked to the surface of the gels, which interact with integrins α5β1 and αvβ3.[6] The endothelial cells grown on the laminin-mimetic gels showed ADAMTS-13, TFPI, tPA, vWF, TF, and P-selectin all increased, with NO increased compared to plate grown cells.[6] The change of gene expression of hemostatic regulators in targeting syndecan binding indicates that syndecans also play an important role in endothelial cell hemostatic regulation. The effects of laminin on endothelial cell hemostasis are shown in Table 7.

6 Summary and Future Directions

This review provides a summary of key interactions of endothelial cells with prominent ECM components of the basal lamina and how these interactions affect endothelial cell hemostatic regulation. Understanding the effects of ECM-endothelial cell interactions provides the potential to design improved blood-contacting materials with long-term thromboresistance. The molecules discussed in this review are focused on the major cellular interactions found between endothelial cells and ECM components of the basal lamina, with more minor and possibly uncharacterized interactions assumed to also be occurring. Further investigation in the area of hemostatic regulation is important because it plays a direct role in thrombotic and embolic complications of cardiovascular devices. It is expected that insight into endothelial cell hemostatic regulation will drive biomedical device design for the reduction of coagulation and increased blood compatibility.

In order to develop an endothelial layer coating with the appropriate hemostatic regulating behavior, the effect of individual and combined integrin interactions should be further elucidated. The studies reviewed here have demonstrated the importance of the substrate on endothelial cell hemostatic regulation, but few have investigated specific binding interactions and the correlated changes in endothelial cell gene expression and thromboresistance. Future investigation would need to isolate integrin interactions on well controlled surfaces for the culture of endothelia cells. Using these platforms it would then be possible to analyze the changes in gene expression as well as the functional changes in endothelial cell hemostatic regulation. Once specific interactions have been correlated to changes in hemostatic regulation, mechanisms and signaling pathways can be elucidated. Understanding signaling pathways and mechanisms could lead to further tailoring of coating designs for endothelialization of biomedical devices. By elucidating the specific effects of integrin attachment on hemostatic regulation, it would then be possible to target specific endothelial cell binding mechanisms to promote a thromboresistant phenotype in an endothelial cell monolayer.

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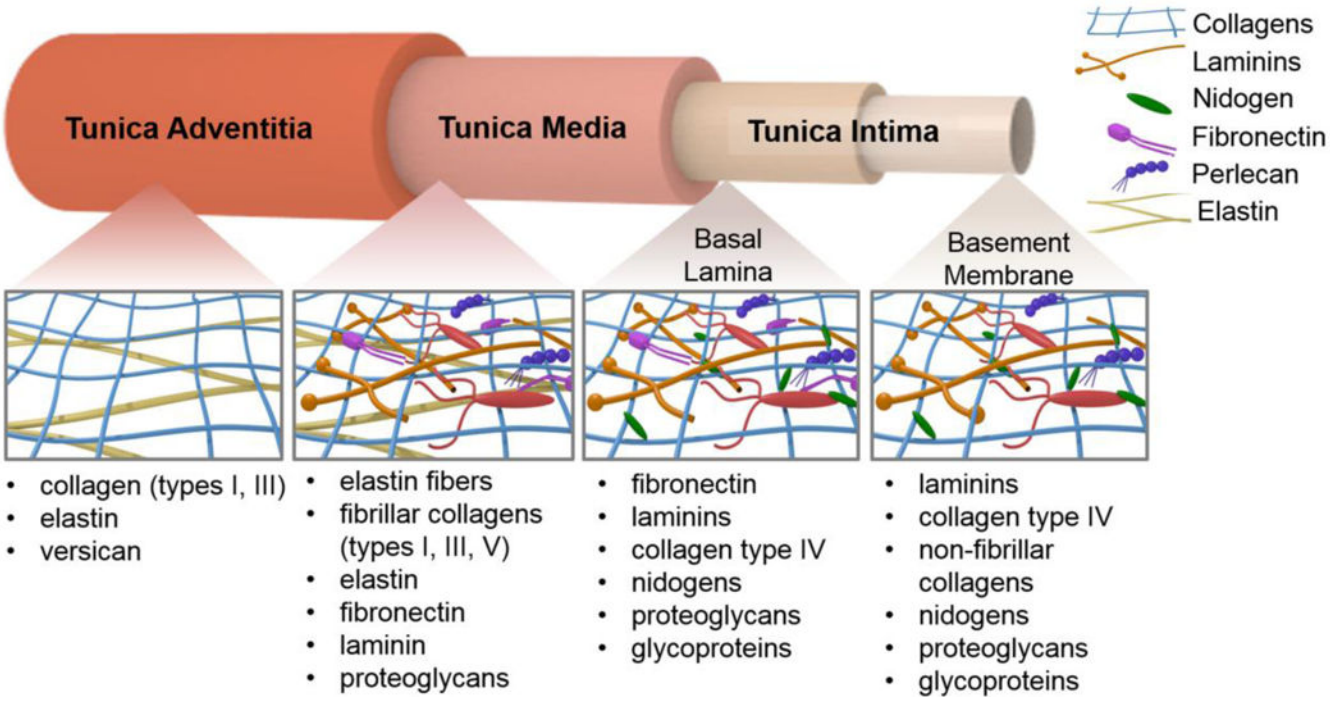

Figure 1:
The vascular layers and their associated extracellular matrix components.

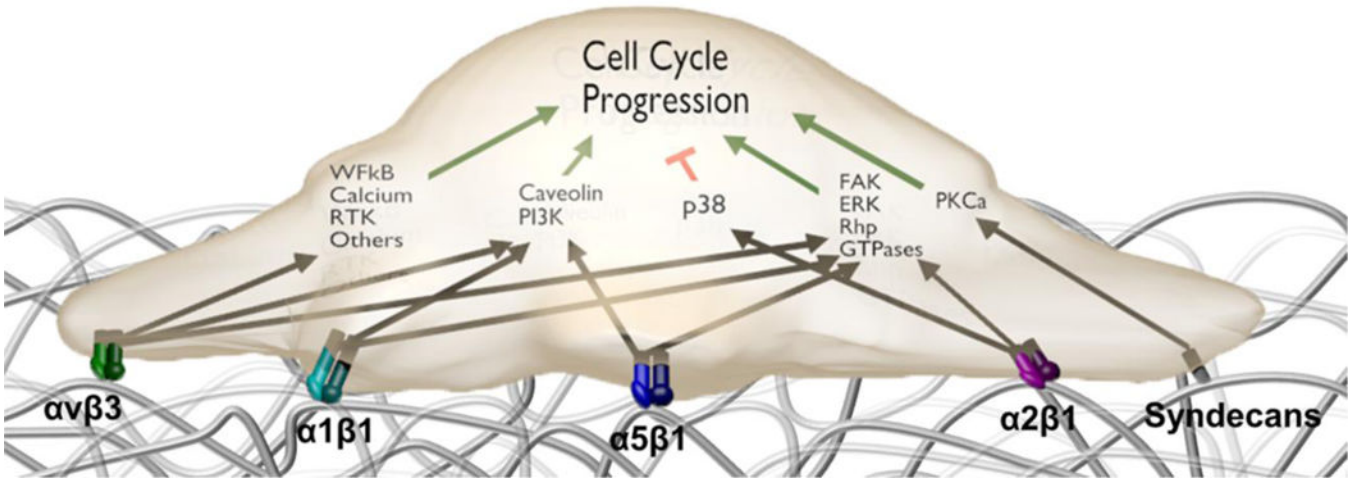

Figure 2:
Common integrin and syndecan signaling pathways in endothelial cells.

Table 1:

Common hemostatic regulators synthesized by endothelial cells in response to environmental cues.

	Protein	Function
Prothrombotic Proteins	Von Willebrand Factor (vWF)	Large multimeric glycoprotein that binds to platelets and proteins during thrombus formation[34]
	Tissue Factor (TF)	Surface protein expressed by activated endothelial cells to initiate coagulation cascade[162]
	Plasminogen activator inhibitor (PAI-1)	Inhibitor of tPA[12]
Antithrombotic Proteins	A disintegrin and metalloproteinase with a thrombospondin type I motif, member 10 (ADAMTS-13)	Enzyme that cleaves vWF[141]
	Tissue factor pathway inhibitor (TFPI)	Major inhibitor of TF, Factor Xa, and thrombin[162]
	Tissue plasminogen activator (tPA)	Regulator of fibrinolysis[12]
	Endothelial nitric oxide synthase (eNOS)	enzyme that catalyzes the production of nitric oxide which inhibits platelet aggregation[161]
	Activated protein C (APC)	Glycoprotein that proteolytically inactivates factor Va and VIIIa to reduce thrombin formation[56]

Table 2:

Summary of the main ECM components found in the vascular wall, their roles, and respective locations.

ECM Component	Role	Location in the Vascular Wall
Collagen	Cell adhesion and signaling; tensile strength	tunica intima (type IV and non-fibrillar), tunica media (types I, III, IV), high relative content in tunica adventitia (types I, III)[160]
Laminin	Cell adhesion and signaling; ECM structural organization	basement membrane[149]
Fibronectin	Cell adhesion and signaling	basement membrane [160]
Nidogen	Crosslinking of other ECM components	basement membrane, basal lamina[148]
Glycosaminoglycans, proteoglycans	Cell signaling; hydration	basal lamina, basement membrane[115, 136]
Elastin	Tissue recoil and elasticity	tunica media[50], tunica adventitia[80]

Table 3:

Summary of integrins, cell expression, and attachment proteins.

Integrin	Proteins with attachment ligands
α1β1	Collagen IV, Colllagen I, Laminin[30, 82, 124]
α2β1	Collagen 1, Collagen IV, Laminin[143, 152]
α5β1	Gelatin, Fibronectin, Fibrillin-1[35, 57, 8, 125]
αvβ1	Laminin, Fibronectin, Osteopontin, Vitronectin[28, 71, 105, 176]
αvβ3	Gelatin, Fibrinogen, Vitronectin, Thrombospondin, Osteopontin, Fibronectin, VEFG-A, Fibrillin-1, vWF[35, 57, 66, 71, 92, 131]
α4β1	Fibronectin[58]
α6β4	Laminin[164]
α6β1	Laminin[21]
αvβ5	Osteopontin, Fibrinogen, Vitronectin, Fibronectin, Thrombospondin[71, 76, 157]

Table 4:

Summary of the syndecan family and their roles as cell attachment mediators.

Syndecan	Cell type	Syndecan receptors
Syndecan-1	Vascular endothelial cells, human umbilical vein endothelial cells, microvascular endothelial cells	Fibronectin, collagen, growth factors[9, 70]
Syndecan-2	Human umbilical vein endothelial cells, microvascular endothelial cells	Fibronectin, laminin, collagen, growth factors[37, 60, 114]
Syndecan-3	Human coronary artery endothelial cells, brain endothelial cells	Matrix molecules *, growth factors[20, 38]
Syndecan-4	Human umbilical vein endothelial cells	Fibronectin, laminin, collagen, growth factors[54, 158]

* Matrix molecule not specified

Table 5:

Summary of the effects of endothelial cell attachment to collagen on hemostatic regulator molecule expression.

Substrate Application	Cell type	Molecules tested	Results
Collagen-derived peptide coat	Human umbilical vein endothelial cell; human aortic endothelial cell	PGI2, vWF; PECAM-1, VE-Cadherin, NOS3, TM, E-selectin;	Increase in NOS3 compared to TCPS; No significant change in PGI2, vWF; PECAM-1, VE-Cadherin, TM, E-selectin[49]
Crosslinked collagen coat	Human umbilical vein endothelial cell; human saphenous vein	PGI2, vWF, tPA, PAI-1	Decrease in PGI2 and vWF secretion; No difference in basal levels of PGI2, tPA increase compared to bare[53, 165]
ePTFE with collagen-1 coat	Human saphenous vein; human umbilical vein	tPA, PGI2, PAI-1	Decrease in PGI2 and tPA; no difference in tPA secretion; PAI increased; Different levels of PGI2, PAI-1 and tPA between unmodified versus modified PTFE[53, 96, 175]
Collagen derived peptide hydrogel (PEG-Scl2)	Human aortic endothelial cell	PECAM-1, VE-Cadherin, NOS3, TM, E-selectin	Decrease in NOS3 and TM on PEG-Scl2 and E-selectin increased compared to collagen; no significant change in PECAM-1[112]

Table 6:

Summary of the effects of endothelial cell attachment to fibronectin and gelatin on hemostatic regulator molecule expression.

ECM Protein	Substrate Application	Cell type	Molecules tested	Results
Gelatin	Gelatin coat on TCPS	Bovine aortic endothelial cell; human saphenous vein endothelial cell	PGI2, PAI, tPA	Increase in NO and PAI; Decrease in PGI2 compared to TCPS[7, 53]
	ePTFE with gelatin coat	Human umbilical vein endothelial cell	PGI2, PAI-1, tPA	Increase in PGI2, PAI-1 and t-PA compared to bare[100]
	PEG-RGD hydrogel	Porcine aortic valvular endothelial cell	ADAMTS-13, TFPI, tPA, vWF, TF, P-selectin	ADAMTS-13, TFPI, tPA, tPA, vWF, TF, P-selectin all increased compared to TCPS[6]
Fibronectin	Fibronectin coat on TCPS	Bovine aortic; human umbilical vein; human saphenous vein	PGI2, vWF, tPA, PAI-1	Increase in PGI2; decreased vWF compared to TCPS; no significant change in $_{tPA}$[7, 53, 165]
	ePTFE with Fn coat	Human saphenous vein endothelial cell; human umbilical vein endothelial cell	tPA, PGI2, PAI-1	Increase in PGI2 and tPA compared to bare ePTFE; no significant change in PAI-1[96, 175]

Table 7:

Summary of the effects of endothelial cell attachment to laminin on hemostatic regulator molecule expression.

Substrate Application	Cell Type	Molecule Tested	Results
Laminin coat on TCPS	Bovine aortic endothelial cell	PGI2	Increase in PGI2 compared to TCPS[7]
PEG-laminin peptide hydrogel	Porcine aortic valvular endothelial cell; human aortic endothelial cell	ADAMTS-13, TFPI, tPA, vWF, TF, P-selectin; NO	ADAMTS-13, TFPI, tPA, tPA, vWF, TF, P-selectin all increased; NO increased compared to TCPS[6, 49]