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Effects of surface properties and bioactivation of biomaterials on endothelial cells

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1. ABSTRACT

Interactions between vascular endothelial cells (EC) and materials are central to biomedical applications such as vascular graft endothelialization or vascularization of an engineered tissue substitute. To improve implant success, biomaterial surfaces are designed to modulate EC adhesion and responses. *In vivo*, EC line all blood vessels; their morphology, function and associated matrix are adapted to and specific for the local microenvironment. To enhance EC adhesion and growth, surface treatments have been developed that modify material surface physico-chemical and mechanical properties. Materials may also be coated with bioactive molecules such as proteins from the matrix, peptides and/or growth factors to study and control EC behaviour. The aim of this review is therefore to give an overview of current knowledge related to EC and their matrix environment *in vivo* and their responses to synthetic surfaces *in vitro*.

2. INTRODUCTION

Adhesion of endothelial cells (EC) on biomaterial surfaces and subsequent responses are of increasing importance in the biomedical field with implication in the endothelialization of vascular grafts or in the formation of a vascular network in engineered tissue substitutes. Prosthetic vascular grafts used to replace small-diameter blood vessels (diameter < 6mm) are characterized by a reduced patency and occlusion: thrombosis and intimal hyperplasia are the main reasons for the high failure (1). The absence of a complete endothelium covering blood contacting devices is a major contributing factor to both phenomena. One approach to prevent thrombosis and to improve the haemocompatibility of synthetic vascular grafts is to create a functional, quiescent monolayer of EC on the graft surface prior to implantation. Another solution is to develop implants that will enhance endothelialization upon implantation (2). EC ingrowth and formation of a

functional, mature vascular network remains a challenge in tissue engineering research. This network is required for the construction or regeneration of many hybrid tissues (1,3). Similarly to normal tissues, many engineered tissue substitutes need blood supply to grow and to remain viable. In addition, implant biocompatibility could be improved by promoting a normal wound healing response including peri-implant vascularization and reduced encapsulation (4).

Biomaterials science and tissue engineering rely heavily on cell-material interactions: surfaces may induce cell adhesion, determine cell fate and then promote the regulated development of functional structures (5). *In vivo*, cells are anchored to their extracellular matrix (ECM) and cell-ECM interactions modulate cell survival and responses (6). Biomaterial surfaces should thus be designed to mimic cell biological environment: the knowledge of the interactions of cells with their natural environment in an organism is essential to develop implants that will be integrated into the host organism.

The objectives of this review are to give an overview of EC and their natural environment *in vivo* and to present surface modifications and their effect on EC response *in vitro*. In the first part of this paper, characteristics and functions of a normal endothelial tissue are presented, and interactions with its ECM *in vivo* are described with a particular interest in the dynamic process of blood vessel formation through angiogenesis. The second part deals with EC interactions with synthetic surfaces. A multitude of surface chemical modifications and bioactive coatings have been developed to promote EC adhesion onto biomaterials. Substrate properties and immobilization mode influence the binding of proteins to their receptors and consequently, cell responses. Combined immobilization of various signalling molecules and the effects on cell responses are also discussed.

3. THE ENDOTHELIAL TISSUE AND ITS MATRIX

Vascular endothelial cells are a specialized type of epithelial cells lining the inner surface of blood vessels of the entire circulatory system, from the heart, arteries and veins to smallest capillaries. They do not form a passive barrier between circulating blood and surrounding tissues. In fact, the endothelium provides a non-thrombogenic surface, communicates with the surrounding microenvironment and releases biochemical regulators. EC hence form a heterogeneous tissue, as they exhibit a great diversity in morphology and functions along the vascular tree depending on vessel type, tissue irrigated and activation state. Interactions between EC and the ECM more particularly are crucial for the maintenance of EC integrity and functions and for the controlled formation and regeneration of blood vessels.

3.1. Morphological and functional heterogeneity

3.1.1. Morphological diversity

Walls of large vessels like arteries and veins consist of three layers: an inner intima made up of a layer of EC attached to their basement membrane, an intermediate media mainly composed of smooth muscle cells (SMC) and elastic fibres and an outer adventitia made of collagenous ECM with fibroblasts, blood vessels and nerves. Arteries are muscular, elastic blood vessels with thick walls that possess elastic laminae surrounding the intima and the media, and they pulsate. Veins have thin walls, they do not pulsate but possess valves (7). *In vivo*, vascular EC experience fluid shear stress, the tangential component of haemodynamic stress. In large straight arteries of uniform geometry, the mean wall shear stress is between 10 to 20 dynes/cm² while in regions of non-uniform geometries (branches and arches) transient shear stress can be as high as 50 dynes/cm² with pulsatile flow (7). EC are thick and aligned in the direction of blood flow in straight segments of arteries but not at branch points. In veins, EC are shorter and flat and are not aligned in the direction of blood flow (7).

Arterioles and venules are intermediate vessels between capillaries and arteries, and capillaries and veins, respectively. Pre-capillary arterioles are completely surrounded by one or two layers of SMC and post-capillary venules are surrounded by pericytes embedded in their basement membrane (8).

Capillary microvessels represent the most abundant vessels in an organism and consist of EC surrounded by a basement membrane and occasional pericytes, allowing direct physical contact between endothelial and tissue cells. EC in capillaries are flattened, elongated, they adapt to their microenvironment and acquire specialized characteristics to accommodate local physiological requirements (7,9,10). Continuous endothelium consists of EC tightly connected to each other via tight junctions and surrounded by a complete basement membrane. It is found in capillaries of the brain, heart, skin and lung, as well as in arteries and veins. Further specialization of the continuous endothelium is observed in blood-brain, blood-retina and blood-testis barriers with acquisition of complex tight junctions and highly regulated transcellular transports. Fenestrated continuous capillaries are characterized by the presence of small openings called fenestrae, and are found in capillaries with an increased fluid exchange between blood and tissues: diaphragmed fenestrated capillaries are found in endocrine and exocrine glands, gastric and intestinal mucosa, renal tubules whereas non-diaphragmed fenestrated are present in renal glomerulus. Finally, discontinuous endothelium, characterized by the presence of many large fenestrations with no diaphragm and gaps, presents a poorly formed basement membrane (discontinuous or absent) and is found in more restricted regions such as capillaries of the liver, spleen and bone marrow.

3.1.2. Functional diversity

Transport function. Capillaries form the main site of exchange of nutrients between blood and tissues. They use several specific transport mechanisms to meet the metabolic needs of the surrounding tissue cells. Fluids and small solutes move passively across the barrier via the paracellular pathway, regulated by intercellular tight junctions, whereas macromolecules use transcellular transports, controlled by the presence of specific membrane

receptors or vesicular carriers such as caveolae and vesiculo-vacuolar organelles (9,11). Spatial heterogeneity of permeability depends on differences in junctional properties and presence or absence of fenestrae and gaps.

Vasomotricity. Transport may be regulated by blood perfusion which is locally controlled by vasomotricity of pre-capillary arterioles. Generally speaking, EC finely control blood flow in response to metabolic demand (oxygen tension and glucose concentration, for instance), cytokines and shear stress, by acting on SMC in vessel walls of arterioles and large vessels. This regulation is short and local, through the production and catabolism of vasoactive molecules by EC, either vasodilators such as nitric oxide (NO), prostacyclin (PGI_2) or vasoconstrictors such as endothelins.

Host defence and inflammation. The endothelium actively participates in the inflammatory response following tissue infection or irritation, mainly at post-capillary venule sites where cell-cell junctions are looser. Activated EC (i) secrete vasoactive molecules to locally increase permeability, (ii) express receptors for immune cell adhesion such as vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) and (iii) secrete cytokines for the recruitment of leucocytes and induction of angiogenesis. EC activation allows adhesion and transmigration of leucocytes to inflammation sites and neovascularization of the injured tissue (9).

Vascular haemostasis. The endothelium lining arteries, veins and all blood vessels provides a non-thrombogenic, anti-coagulant surface by the secretion and/or surface expression of several regulatory factors that maintain blood in a fluid state. An intact EC monolayer is covered by a layer of glycocalyx containing anti-coagulant heparan sulphate proteoglycans (HSPG) and anti-thrombic thrombomodulin. EC also secrete vasodilators that prevent platelet adhesion. When a vascular lesion occurs, platelets adhere to exposed vessel walls, EC and surrounding cells express or secrete pro-coagulant molecules (e.g., platelet-activating factor and P-selectin) leading to the formation of a fibrin clot and finally EC produce fibrinolytic effectors to limit clot formation. The nature of factors involved in vascular haemostasis depend on location in the vascular tree (11).

3.2. Endothelial cell - matrix interactions

3.2.1. Cell-ECM interactions

EC interactions with their underlying ECM are essential for maintenance of cell integrity and functional activity, and for the formation of functional mature blood vessels. The ECM provides mechanical support and biochemical cues for cell adhesion, migration, proliferation and differentiation via interactions with cell membrane receptors and through growth factor sequestering. Matrix proteins and more particularly adhesive ones such as fibronectin (Fn), laminins (Ln) and vitronectin (Vn) possess many binding domains capable of interacting with other ECM proteins as well as with cell surface receptors. Defined amino acid sequences present within ECM molecules specifically bind cell surface receptors to trigger various intracellular pathways. Cell-ECM adhesions are mediated primarily by integrin receptors, heterodimeric transmembrane proteins composed of α and β subunits that connect the ECM molecules to the cell cytoskeleton. When bound to their specific ligand, integrins cluster, form focal adhesion structures, mediate cell anchorage to the underlying matrix, and can also initiate signalling cascades transduced to the nucleus. These events may affect many aspects of the cell responses such as proliferation, differentiation, migration and survival (12). A single cell binding motif can be found within several proteins, such as the most investigated Arg-Gly-Asp (RGD) sequence present in Fn, Vn and Ln among others. A protein is able to bind several receptors through various sequences which exposition depends on protein self-assembly into fibres or a network, its association with other ECM molecules or its proteolytic degradation. Moreover, cell membrane receptors frequently associate with other receptors such as integrins or growth factor receptors, allowing integration of diverse signalling pathways. Hence, signals transduced to cell nucleus depend on the set of membrane receptors expressed by cells, as well as on the composition, structure and spatial organization of the underlying ECM which are characteristic of a tissue at a given time (6).

3.2.2. Vascular basement membrane

Basement membranes are specialized types of ECM, highly cross-linked and organized in a sheet-like structure that separate the epithelium from the connective tissue. They function as selective filters, maintain mature tissue function and define spatial organization during tissue development and reconstruction following tissue injury, by regulation of cell growth, differentiation, and migration (13). The upper layers, called basal lamina, are secreted by epithelial cells and consist of a network of Ln and a network of collagen type IV interconnected via nidogen/entactin. The lower layer of the basement membrane is secreted by cells from the underlying connective tissue and contains fibrils of collagen type I and type III and Fn (13,14).

The Ln network assembly is necessary for the basal lamina formation and plays an essential role in cell adhesion and signalling (14). The laminins are a family of heterotrimeric molecules composed of α-, β- and γ-chains. Ln α-chains, in particular, possess many receptor binding sites and they are expressed in a tissue-specific and developmentally regulated manner, conferring heterogeneity among basement membranes (13,15). EC express only 2 Ln α-chains: Ln α4 which is a component of Ln-8 ($\alpha4\beta1\gamma1$) and Ln-9 ($\alpha4\beta2\gamma1$) and α5 which is a component of Ln-10 ($\alpha5\beta1\gamma1$), Ln-11 ($\alpha5\beta2\gamma1$) and Ln-15 ($\alpha5\beta2\gamma3$). Ln α4 is the predominant α chain found in vascular basement membranes and is expressed by all types of EC, both during development in the embryo and in the adult, while Ln α5 is detectable in basement membranes of quiescent mature vessels, primarily in capillaries and some venules after birth and is not associated with a fenestrated endothelium (16,17). Ln-10 is believed to be involved in vessel maturation and stability (16,18). EC bind to the Ln network via integrin receptors including integrins $\alpha3\beta1$ and

α6β1 for both Ln α4 and α5 chains and αvβ3 and αvβ5 for Ln α5 chain via exposed RGD sites. Ln α5 also binds the α-dystroglycan and the Lutheran blood group transmembrane glycoproteins (16,19).

Collagen type IV network provides the scaffold mechanical resistance (13). In addition, network forming collagen type VIII, closely associated with human vascular basement membranes and therefore used as a marker for blood vessels, maintains vascular basement membranes in an open porous structure (16). Ln and collagen IV networks are principally linked by the nidogen/entactin-2 isoform (16).

Proteoglycans (PG), proteins with glycosaminoglycan side chains, associated with basement membranes play a structural role in maintaining tissue architecture via interactions with matrix proteins, help in selective filtration, sequester soluble growth factors via their heparan sulphate (HS) side chains and thus help in regulating cell differentiation (13,14). In addition to perlecan, agrin and collagen type XVIII, heparan sulphate proteoglycans (HSPG) associated with basement membranes, chondroitin sulphate PG such as leprecan and collagen type XV are detected in vascular basement membranes (14-16). Vascular endothelial cells synthesize HSPG, including perlecan (11).

Thrombospondins (Tsp) belong to a group of proteins called matricellular which interact with cell receptors and matrix components. They are defined as regulators of cell function and are expressed at high levels during development and in response to injury (20). Tsp-1 and -2 are strong inhibitors of angiogenesis and are both involved in pathological conditions. However, only Tsp-2 is associated with developing vessels (16,20). Another molecule of interest is the von Willebrand factor, a thrombogenic molecule released by EC that favours platelet adhesion, and which is primarily found in basement membranes of veins (11). Finally, as EC in capillaries may be in close contact to surrounding tissue cells, they interact with composite basement membranes containing other isoforms including Ln α-chains produced by surrounding cells (16).

3.3. Formation of blood vessels

3.3.1. Context and initiation

Blood vessels in the embryo develop through vasculogenesis i.e., through *in situ* differentiation of mesodermal precursor cells, called angioblasts, into EC that assemble into a primary capillary plexus. The primitive network is then developed, remodelled and stabilized through the process of angiogenesis into a complex, mature, and functional network (21). In the adult, EC interact with a laminin-rich ECM that maintains mature vessel in a stable quiescent state. During regulated physiological processes such as vascularization or wound repair, EC undergo rapid proliferation to form new vessels following matrix remodelling via sprouting. Activated EC degrade the underlying basement membrane, migrate and proliferate in the perivascular matrix, form tubular structures that become mature and functional (22). Under these conditions, angiogenesis is transitory and highly regulated, spatially and temporally. However, many diseases such as arthritis, diabetes and tumour growth are driven by a persistent unregulated angiogenesis. Thus, control of the angiogenic process is essential and relies especially on regulated cell-matrix interactions. Gene knock-out experiments in mice and *in vitro*/*in vivo* experiments allowed to define a mechanism of sprouting angiogenesis.

Local hypoxia, hypoglycaemia, shear stress or inflammation induces the release of pro-angiogenic vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)-2 that in turn attract EC (18,22-24). Activated EC locally increase vascular permeability thereby allowing extravasation of plasma proteins that lay down a provisional matrix rich in fibrin and fibronectin. They also secrete proteinases such as plasminogen activators (PA) leading to the generation of plasmin and matrix metalloproteinases (MMP) that degrade ECM proteins and liberate growth factors sequestered within the ECM. Angiopoietin (Ang)-2, a growth factor antagonist of the Tie2 receptor, destabilizes existing vessels probably by loosening EC adhesion with local basement membrane and periendothelial cells that surround and support blood vessels (21).

3.3.2. Proliferation and migration

Local ECM remodelling not only creates a free path for EC to migrate towards the angiogenic stimulus, but also induces the participation of a new set of cell-matrix interactions, eliciting new signals. The release of matrix-bound growth factors such as FGF-2 and heparin-binding forms of VEGF induces angiogenic signals promoting EC proliferation and migration and modulates cell integrin expression (23-25). Matrix protein cleavage or conformational changes following remodelling exposes cryptic sites within matrix proteins that alter their function promoting EC proliferation and migration (25). Collagen type I and type IV usually interacts with α1β1 and α2β1 integrins whereas cleaved molecules expose cryptic sites interacting with αvβ3 (15). Proteolytic degradation also induces production of soluble fragments such as endostatin, from collagen type XVIII, that can exert anti-angiogenic effects by inhibiting EC proliferation and migration and thus permit control of the angiogenic process (25). Finally, EC previously exposed to a laminin-rich stabilizing ECM, then interact with a new set of ECM molecules from the fibronectin-rich provisional matrix, enhancing proliferation. Formation of new blood vessels in embryos and in adult organisms relies upon different endothelial integrins and ECM ligands: in the embryo, a successful vascular development depends on Fn and its major receptor α5β1, but not on αvβ3 and αvβ5, which are up-regulated in adult and pathological angiogenesis (18,24,26). Non-proliferative cells located at migrating tip are exposed to the interstitial matrix rich in collagen type I and type III fibres (24). Concurrently, EC reorganize into cord-like structures, which acquire a lumen and form interconnected tubes (17).

3.3.3. Stabilization and maturation

Vessel mature or regress depending on their use in the network, which is modeled by blood flow generated forces. Haemodynamic forces induce modifications of cell-cell and cell-ECM adhesions and are believed to up-regulate growth factors such as platelet-derived growth factor (PDGF)-BB (8,22,27). EC recruit PDGF receptor-α-expressing periendothelial cells and contact between EC and mural cells triggers the activation of transforming growth factor (TGF)-β that inhibits EC proliferation and migration, induces SMC differentiation, stimulates basement membrane production and deposition and alters integrin profiles (8,18,22,23,27). Ang-1, a Tie2 ligand, stabilizes EC-EC interactions and adhesions of mural cells with EC (21,23). Mural cells adhesion and deposition of a complete stable basement membrane provide stability against rupture or regression, in absence of VEGF except for a fenestrated endothelium (23). EC interaction with periendothelial cell is essential for deposition of a complete stable basement membrane.

Further vessel specialization is realized by EC interaction with repelling cues for arterio-venous determination and guided vessel branching, heterotypic interaction with periendothelial cells such as astrocytes involved in the blood-brain barrier formation, interaction with organ-specific growth factors such as endocrine gland (EG)-VEGF for endocrine gland capillaries specialization, and other non determined processes (7-10).

4. INTERACTIONS BETWEEN ENDOTHELIAL CELLS AND BIOMATERIAL SURFACES

Endothelial cell adhesion on biomaterial surfaces is required to provide a non-thrombogenic surface to vascular prosthesis, to subsequently induce formation of blood vessels around implanted biomedical devices improving their biocompatibility or to promote vascularization of growing (hybrid) tissues for regenerative medicine. Cell-material interactions must then incite EC adhesion, but also allow cells to maintain their differentiated functional state, and in some cases guide sprouting i.e., regulated cell proliferation and migration.

The ability of a material to support cell adhesion depends on its surface properties. Treatments to modify a surface chemistry or topography have been used to induce protein adsorption and subsequent cell adhesion. Surface attachment of bioactive molecules such as ECM proteins, adhesive peptide sequences and/or growth factors have been used to promote EC adhesion onto materials and to control cell processes such as proliferation, migration, differentiation and survival.

4.1. Surface properties and the mechanical environment

4.1.1. Physico-chemical properties

Cell attachment to synthetic surfaces relies heavily on the presence of adsorbed proteins from media containing serum, blood plasma or cellular secretion of matrix proteins. The physico-chemical properties of the surface (e.g., molecular architecture, chemical composition, charge) determine the composition of the adsorbed protein layer as well as the amount and conformation of adsorbed proteins. Protein adsorption results from a combination of interactions with material surfaces including hydrophobic interactions, electrostatic forces, hydrogen bonding and van der Waals forces (28).

It is generally observed that EC adhere and spread on hydrophilic surfaces such as tissue culture polystyrene (TCPS) and glass, while EC adhesion is reduced or even absent on hydrophobic surfaces such as polytetrafluoroethylene (PTFE), polyethyleneterephthalate (Dacron) and fluoroethylenepropylene (FEP) (29-31). Differences in surface hydrophilicity result in quantitative and qualitative variations in the composition of the adsorbed protein layer. Proteins preferentially bind to different surfaces dependent on their nature, and adsorption onto a surface induces protein conformation changes, more or less important, depending on the surface properties (32). Hydrophobic surfaces can exert strong attraction with hydrophobic parts of the protein (inside); some believe that proteins strongly and usually irreversibly adhere to these surfaces and may undergo denaturation i.e., disruption of native conformational state, altering exposition of cell binding sites naturally exposed on the outside of the protein. When materials are exposed to blood plasma or serum, albumin strongly binds to hydrophobic surfaces while conformationally active adhesive proteins such as Fn and Vn preferentially adsorb on hydrophilic surfaces (33,34). Some researchers believe that as adsorption onto hydrophilic surfaces is reversible, proteins can be displaced (29).

Many polymeric biomaterials used for clinical applications (vascular grafts) are hydrophobic (PTFE, Dacron, FEP, polyurethanes). Some believe that a solution to promote cell adhesion is to increase surface hydrophilicity by chemical treatments (UV exposition, alkaline hydrolysis) or plasma treatment (ammonia or oxygen plasma treatment), all resulting in the addition of polar groups and charges (35-37). TCPS and Primaria, routinely used for cell culture, have been made hydrophilic and cell adhesive by plasma treatment. Since, EC adhesion to hydrophobic polymeric surfaces has been enhanced by plasma modification using either nitrogen- or oxygen-containing monomers: modified surfaces are more hydrophilic, adsorb more adhesive proteins such as Fn and support cell adhesion, spreading and growth (38-41). Moreover, EC adhering on plasma-treated surfaces show improved anti-coagulant and fibrinolytic activities and better resist to shear stress induced detachment (38,40).

Surface exposition of various chemical groups and charges affect initial cell adhesion. In presence of serum, initial cell attachment on nitrogen-rich surfaces such as Primaria is a result of adsorption of Vn and Fn, while cell adhesion on oxygen-rich surfaces such as TCPS is mediated by adsorbed Vn only, as Fn adsorption on these surfaces is sub-optimal for cell adhesion (39,40,42,43). Oxygen-rich surfaces present negatively charged groups while nitrogen-rich surfaces rather expose positively charged groups (at physiological pH). Fn is an acidic protein overall negatively charged that more abundantly

adsorbs on positively charged surfaces. Introduction of electrical charges on a surface can enhance protein adsorption via electro-attractive forces. Addition of a polyelectrolyte film on a poorly adhesive surface, either as a monolayer or a multilayer film resulting from alternate adsorption of polycations and polyanions, enhances cell attachment (44,45). In case of weak polyelectrolyte gels, EC adhesion increases with charge density while cells highly adhere and proliferate on strong polyeletrolytes, they also expose higher amount of anti-platelet HSPG and are more resistant to shear stress than confluent EC attached on glass or TCPS (44,46,47).

However, correlating surface hydrophilicity with cell responses is very risky as no clear trend can be made between biomaterial water wettability and cell behaviour (Evan Dubiel, Yves Martin, Patrick Vermette, manuscript in preparation).

4.1.2. The mechanical environment

Cells sense and respond to underlying substrate stiffness, to topography and to fluid flow. External mechanical forces can be sensed by cytoskeleton-linked receptors or the cytoskeleton structure itself. The mechanical coupling of the cytoskeleton with cell-ECM and cell-cell adhesions allows transduction of mechanical signals throughout the cell (48). Upon adhesion, cells form adhesive contacts with surfaces and pull on the substrate. Increased surface stiffness is associated with increased cell contractility (49). On stiff surfaces, cells have an increased focal adhesion formation and a more organized cytoskeleton with formation of actin stress fibres, resulting in cell spreading and a higher adhesion strength than on soft substrates (45,49,50). Substrate stiffness and cell contractility appear to regulate EC proliferation and differentiation: EC form tube-like structures on soft malleable substrates while they proliferate on rigid surfaces (45,51,52).

EC are also sensitive to surface topography (53). When attached to a surface presenting micro- or even nano-features, they emit filopodia and lamellipodia, sensory organs of cells, and undergo morphological changes (54). On grooved surfaces, EC elongate and align parallel to the channel direction; cell orientation persists until near confluence is reached and increases with channel depth (55-57). Cell actin stress fibres and focal adhesions align parallel to channel direction and focal contacts are preferentially located at feature edges (56). Their localization correlates with preferential Fn fibrils formation at edges of grooves, pillars or wells (58). However, enhancement of cell elongation and orientation associated with actin stress fibre alignment has also been observed on wave features, in absence of sharp edges (59). Grooved and waved topographies induce polarization of cell movement: cell orientation and directed movement in response to substrate topography may be referred to as “contact guidance” (53). Random topography or increased surface roughness have been shown to enhance EC adhesion but also Fn and Vn adsorption (60,61). Hence, surface roughness and topography may affect cell morphology directly by directing focal adhesions and stress fibres assembly, but they may also influence cell behaviour indirectly by altering surface protein adsorption.

The shear stress applied to the luminal surface of cells can be sensed by the cell membrane and the associated receptors and this stress can be transmitted throughout the cell to cell-matrix and cell-cell adhesions. As a result, shear stress induces EC alignment and an increase of stress fibres and remodels cell-matrix adhesions to increase adhesion strength. In confluent EC, shear stress increases the size of focal adhesions and activates integrins such as $\alpha v\beta 3$ and $\alpha 5\beta 1$ (48). In non confluent EC, shear stress promotes cell spreading, then lamellipodial extension in the flow direction, cell-cell adhesion dissociation and enhances directed cell migration. The existing focal adhesions under the main cell body increase in size while new transient focal adhesions assemble under the lamellipodia and align with the flow direction (48,62). Shear stress preconditioning may be used to orient EC or to increase cell adhesion strength to the substrate (63).

4.2. Pre-coatings made of matrix proteins

4.2.1. Protein coatings

Surface treatment may enhance cell adhesion by modifying protein adsorption. However, the exact composition of the adsorbed protein layer is not known and cannot be controlled or reproduced. Moreover, surface modification may not be sufficient for the formation of a confluent endothelial monolayer. Hence, individual components of the ECM such as Fn, collagen type I and IV have been used as surface coatings to facilitate cell attachment onto biomaterials and to promote their biocompatibility (29,34). The most used and studied coating consist in an adsorbed or immobilized layer of Fn which strongly enhance EC adhesion and spreading, the formation of focal adhesions, and the organization of actin filaments into stress fibres, via interaction with its main receptors, integrins $\alpha 5\beta 1$ and $\alpha v\beta 3$. EC anchored to Fn proliferate, migrate, resist shear stress induced detachment and keep their differentiated phenotype (29,64). Vn and fibrinogen, both $\alpha v\beta 3$ ligands, enhance EC adhesion, spreading and motility (25,65,66). Collagen type I or I/III and collagen type IV promote EC adhesion and retention and induce EC migration to a higher extent than Fn via $\alpha 1\beta 1$ and $\alpha 2\beta 1$ (65,67-70). Laminins also support EC attachment and spreading but to a lesser extent than Fn, collagen and Vn (65). EC adhere more strongly and spread on Ln-10 rather than Ln-1 or Ln-8, with no formation of focal or even fibrillar adhesions, indicative of a motile phenotype (19,71). Ln-10 also better supports EC migration (71).

Osteopontin and tenascin, two matricellular proteins mainly detected during development and in response to tissue injury i.e., associated to remodelling, promote EC adhesion and migration via $\alpha v\beta 3$ integrin in particular (20,25,72). On the contrary, Tsp and SPARC/osteonectin are anti-adhesive proteins, they induce an intermediate state of adhesion associated with a disruption of focal adhesions formation; they inhibit migration and VEGF-induced proliferation (20).

Table 1. Effects of ECM molecules and growth factors on endothelial cells *in vivo*

Molecules	Effects on endothelial cells (EC)	Ref.
Molecules from the ECM		
Fibronectin (Fn)	• Present in the lower basement membrane of mature vessels or in the provisional matrix of developing vessels. • Enhances EC proliferation and migration via interaction with its main receptors: integrin α5β1 (embryo angiogenesis), integrins αvβ3 and αvβ5 (adult and pathological angiogenesis).	16, 18, 24, 26
Laminin (Ln)	• Laminins are essential components of the upper basement membrane (basal lamina) of endothelia. • EC express only Ln α4- and Ln α5-chains: o α4-chain (Ln-8 and Ln-9): found in basement membranes underlying all types of EC. o α5 chain (Ln-10, Ln-11 and Ln-15): found in basement membranes of quiescent mature vessels. • The Ln network provides signals such as adhesion and maintains mature vessels in a stable quiescent state. • EC integrin receptors for Ln: o α3β1 and α6β1 for both Ln α4 and α5 chains. o α6β4, αvβ3 and αvβ5 are potent α5-chain receptors.	13-19, 21
Collagen I and III	• Fibrous collagen types found in the lower basement membrane. Provide a non-proliferative signal, stabilize vessel walls. • Interact with α1β1 and α2β1 integrins. Collagen type I binds integrin αvβ3 when cleaved.	15, 24, 25
Collagen IV and VIII	• Network forming collagen types. Collagen type IV is an essential component of the basal lamina, its network provides mechanical resistance. • Collagen VIII is specifically expressed in vascular basement membranes. • Collagen type IV interacts with integrins α1β1 (mainly) and α2β1 and with integrin αvβ3 when cleaved.	13, 15, 16
Heparan Sulfate ProteoGlycans (HSPG)	• Components of the basement membrane. Have a role in filtration and growth factors sequestering. Also act as co-factors for growth factors binding to their receptors. • Main vascular HSPG: perlecan, agrin, collagen XVIII.	13-15, 17
Thrombospondins (Tsp-1 and Tsp-2)	• Matricellular proteins associated with the basement membrane, inhibitors of angiogenesis. • Tsp-2 is associated with developing blood vessels.	16, 20, 25
Endostatin (soluble)	• Collagen type XVIII proteolytic fragment. • Specifically inhibits EC proliferation and migration, inhibits angiogenesis.	25, 140
Growth factors		
Vascular endothelial growth factor (VEGF)	• Growth factor specific for EC via its receptors VEGF-R2 and VEGF-R1. • Pro-angiogenic action. Increases vascular permeability, promotes EC proliferation and migration.	18, 22-24, 141
Angiopoietins (Ang-1 and Ang-2)	• Growth factors specific for EC via their receptor Tie2. • Ang-1 (Tie2 ligand) stabilizes the mature vasculature (intercellular interactions). • Ang-2 (Tie2 antagonist) acts as a destabilizing signal: induces vessel regression in absence of VEGF and sprouting in presence of VEGF.	21, 23, 142
Fibroblast growth factor-2 (FGF-2), also known as basic FGF (bFGF)	• Angiogenic factor. Promotes cell proliferation and migration.	18, 22-24
Transforming growth factor-beta (TGF-β)	• Involved in the maturation phase of angiogenesis, can act as a regulator of cell proliferation, migration, survival, differentiation, and ECM synthesis in EC and SMC. • TGF-β can be a stimulator and an inhibitor of angiogenesis depending on the activation of its receptors, the EC-restricted ALK1 and the broadly expressed ALK-5, which have opposite effects on EC behaviour.	8, 18, 21-24, 27, 143

EC stands for vascular endothelial cells, ECM for extracellular matrix, SMC for smooth muscle cell. Molecules from the ECM are in their immobilized form unless stated otherwise, growth factors in their soluble form.

The nature of the coated protein and the various expressed integrins may differentially regulate intracellular signalling. Cell attachment to a surface and subsequent behaviour also depend on protein density, conformation and spatial organization which, in turn, depend on its interaction with the underlying substrate.

Tables 1 and 2 list the effects of ECM molecules and growth factors on EC *in vivo* and *in vitro*, respectively.

4.2.2. Protein-surface interaction and cell adhesion

Collagen type I molecules self-assemble into fibrillar structures or into a network only when adsorbed on hydrophobic surfaces (73,74). EC adhering to collagen fibrils have a reduced spreading and a weak cytoskeleton organization corresponding to a motile phenotype, while EC on a collagen type I network spread and present actin stress fibres, indicative of strong adhesion (74). Furthermore, collagen type IV network formation on a hydrophobic surface enhances EC adhesion and retention (68). Collagen spatial rearrangement may expose or hinder cell binding sites, thus changing the availability of amino acid sequence, density and spatial distribution of exposed motives. In the same way, Fn adsorbed on a hydrophilic surface supports higher EC adhesion than when adsorbed on a hydrophobic surface and enhances cell retention (64,75). The protein exposes a higher density of cell binding sites when exposed to a hydrophilic substrate, consequently, cells may form more bonds with the underlying surface resulting in a higher adhesion strength (75-77). Again, surface hydrophilicity does not correlate well with cell responses (Evan Dubiel, Yves Martin, Patrick Vermette, manuscript in preparation).

Cell adhesion studies on hydrophilic model surfaces exposing neutral, positively charged or negatively charged groups and precoated with Fn showed that chemistry alter Fn conformation and strongly influences

Table 2. Effects of surface-bound ECM molecules, peptides, and growth factors on endothelial cells *in vitro*

Molecules	Effects on endothelial cells	Ref.
ECM proteins		
Fibronectin (Fn)	• Enhances EC adhesion, spreading, proliferation, migration via its binding to integrins α5β1 and αvβ3 with different affinity levels. • EC responses depend on Fn conformation, orientation and on its strength of attachment to the surface i.e., on the surface properties of the underlying substrate. ○ When exposed to soluble FGF-2, EC adhering to Fn can proliferate and form a confluent monolayer or rearrange their matrix into cords and form capillary-like structures depending on Fn anchorage strength.	29, 34, 36, 64, 75, 76, 78-80, 82, 86-89
Laminin (Ln)	• Laminins support EC attachment and spreading, but to a lesser extend than Fn, collagen, and Vn. • Ln-10 supports EC migration and stronger adhesion and spreading than Ln-1 and Ln-8, via interaction with integrins α3β1 and α2β1.	19, 65, 71
Collagen I/III Collagen IV	• Interact with α1β1 and α2β1 integrins. • Facilitate cell attachment and induce EC migration (> Fn). • Cell responses depend on spatial rearrangement and thus on the surface properties of the underlying substrate. ○ EC adhering to collagen type I fibrils show a motile phenotype, while EC on a collagen type I or type IV network show strong adhesion and resist shear stress.	65, 67-70, 74, 144
Vitronectin (Vn) Fibrinogen	• Enhance EC adhesion and motility. • Bind integrin αvβ3 ligand.	25, 65, 66
Osteopontin Tenascin	• Enhance EC migration. • They interact with integrin αvβ3.	20, 25, 72, 88
Thrombospondins (Tsp-1 and Tsp-2)	• Anti-adhesive proteins. • Inhibit EC migration and VEGF-induced EC proliferation.	20
Peptides		
RGD	• The most effective and ubiquitous cell adhesive signal. • Cell response depends on RGD concentration, clustering and peptide conformation. • Linear RGD sequences such as GRGDSP preferentially mediate cell adhesion via integrin α5β1. • Cyclic RGD or kinked sequences such as GRGDY or GRGDVY selectively bind integrin αvβ3. They specifically promote EC and SMC adhesion.	91, 96-101, 110, 111, 113
REDV	• Sequence from the Fn CS5 domain. • Selectively binds α4β1 integrin, specifically promotes EC adhesion. • The pro-adhesive action is more reliable when the entire CS5 domain is used.	102-104, 123
SVVYGLR	• Sequence exposed after proteolytic cleavage of osteopontin. • Mediates EC adhesion and migration via a receptor expressed by few cell lines, integrin α9β1.	105
YIGSR	• Issued from the Ln β1-chain. • Promotes cell adhesion with no formation of focal adhesions or actin stress fibers. • Combined with RGD, leads to an increased EC migration.	90, 102, 108
PHSRN	• From Fn, RGD synergy site for integrin α5β1 binding: does not support cell adhesion on its own but acts synergistically with RGD to increase α5β1 binding, cell adhesion and spreading.	90, 99, 100, 117
WQPPRARI and SPPRRARVT	• Sequences issued from Fn C-terminal heparin binding domain II. Bind syndecan-1 and -4. • Support cell adhesion with formation of focal adhesions and actin stress fibers. • Combined with RGD enhance EC proliferation and migration.	109, 118, 119
Growth factors		
Vascular endothelial growth factor (VEGF)	• Soluble VEGF: binds VEGF-R2, promotes EC proliferation and migration. Enhances integrins αvβ3 and α5β1 activation. • Immobilized VEGF: binds integrins αvβ3, α3β1 and α9β1, mediates EC adhesion and migration. • Co-immobilized with Fn, enhances EC proliferation.	21, 70, 128, 129
Fibroblast growth factor-2 (FGF-2)	• Soluble FGF-2 binds FGF-receptor and integrin αvβ3. It promotes EC proliferation and migration. • Immobilized FGF-2 promotes EC adhesion via its interaction with integrin αvβ3. Induces EC migration and prolonged proliferation via its receptor.	72, 127, 130, 131
Hepatocyte growth factor (HGF)	• Immobilized promotes prolonged EC proliferation compared to soluble HGF.	132
Angiopoietins (Ang-1 and Ang-2)	• Immobilized Ang-1 and Ang-2 promote EC adhesion, at least in part via integrins. • Only Ang-1 enhances EC spreading, focal adhesion formation, and migration.	133

subsequent integrin binding. Depending on surface chemistry, Fn binds with different affinity levels α5β1 and/or αvβ3 integrins. Accordingly, focal adhesions assembly, composition and then elicited signals differ depending on surface chemistry and Fn conformation (78-80). Furthermore, Fn adsorbed onto hydrophilic negatively charged surfaces is weakly bound and can be displaced. The protein is then rearranged into fibrils by EC via interaction of its N-terminal domain with cellular α5β1 (81). Fn fibrils formation is associated with formation of fibrillar adhesions rich in α5β1 integrin and an increased assembly of focal adhesions. On the contrary, Fn covalently attached to a surface or adsorbed to mild hydrophobic or positively charged surfaces strongly interacts with the substrate and cannot be rearranged into fibrils by cells. EC attached on strongly bound Fn present fewer focal

adhesions than when anchored to weakly bound Fn (36,82). Similarly, fibroblasts adhering on a strongly bound Fn layer cannot rearrange Fn into fibrils, they present focal contacts rich in α5β1 and show an increased adhesion strength associated with an increased Fn-α5β1 bonds density (83,84). On the other hand, fibroblasts exposed to weakly bound Fn form fibrillar adhesions associated with Fn fibrils and focal adhesions rich in αvβ3 integrin and they present a higher motility (84,85). Hence, integrin binding to Fn result in different cell behaviour depending on substrate pliability. Similarly, when exposed to FGF-2 for a few days, EC attached on weakly bound Fn rearrange their Fn matrix into cords and form capillary-like structures, while EC anchored to Fn strongly interacting with the underlying substrate proliferate and form a confluent monolayer resistant to shear stress detachment (36,64). Then, formation of capillary-like structures from EC on a surface seems to be related to cell anchorage strength to surface that in turn depends on the matrix rigidity as well as on its biochemical composition.

Strategies to immobilize and orient proteins in order to favour ligand-integrin binding and then cell adhesion strength have been developed. Most current protein immobilization procedures involving covalent chemical coupling result in a random distribution of protein orientations on the surface, leaving a significant fraction of binding sites inaccessible to cells, and may be responsible for loss of protein biological activity. Since, an intermediate layer of antibodies or peptides directed towards a region away from the Fn cell binding site have been used to orient Fn molecules (86,87). An alternative approach is to take advantage of natural interactions between ECM molecules to both bind and orient a biomolecule naturally, thereby enhancing its biological activity. Osteopontin naturally binds collagen type I and adsorption of osteopontin on a collagen I intermediate layer enhances EC adhesion (88). Likewise, Fn naturally interacts with HSPG in the matrix and Fn interaction with heparin, a natural analog of HSPG, increases exposition of its cell binding and VEGF binding sites (76,89), enhancing VEGF-induced EC proliferation and migration.

4.3. Grafting of peptides

4.3.1. Sequence and receptor selectivity

Since the discovery of amino acid sequences within ECM proteins specifically recognized by cell receptors, many researchers have immobilized cell recognition peptides directly onto material surfaces in order to control elicited signals and subsequent cell behaviour. In addition to providing binding specificity, peptides present the advantage of being conformationally stable, easy to synthesize and to modify. They usually are firmly linked to surfaces either directly or via a spacer arm to enhance their steric availability and conformational freedom, thus promoting their binding. Control of the desired signal and subsequent cell responses are better achieved if there is minimal non-specific protein adsorption. For that purpose, peptides can be immobilized on a low-fouling layer that resist (or at least limit) non-specific protein adsorption. These layers can be made of poly(ethylene glycol) (PEG) (also often referred to as poly(ethylene oxide)) layers (90-92), polysaccharides layers (93-97), and phospholipid bilayers (98-100).

RGD is the most effective and most often used peptide sequence to promote adhesion on synthetic surfaces (101). The RGD sequence was first identified in the 10th Fn type III repeat (FnIII-10), then in other matrix proteins such as Vn, Ln and collagen. Many integrins bind molecules in a RGD-dependent manner such as integrins α5β1 and αvβ3, which are predominant cell adhesion mediators. Amino acids flanking the RGD sequence and conformation of the amino loop are mainly responsible for their different integrin affinity and selectivity. Cyclic RGD peptides or kinked sequences such as GRGDY and GRGDVY selectively bind the αvβ3 integrin while linear RGD sequences such as GRGDSP preferentially mediate cell adhesion via α5β1 (99-101). Cell integrin expression pattern determines the relative adhesion strength on a peptide surface. EC, SMC and fibroblasts equally adhere and spread on linear RGD sequences while cyclic RGD peptides preferentially mediate EC and SMC adhesion via αvβ3. Concerning integrin selectivity, some other non-RGD signal recognition sequences are used in integrin binding. REDV peptide derived from Fn alternatively spliced CS5 domain selectively addresses integrin α4β1 expressed in a small number of cell lines. REDV specifically mediates adhesion of EC but not SMC nor fibroblasts via α4β1 (102-104). The SVVYGLR sequence, exposed after proteolytic cleavage of osteopontin, mediates EC adhesion and migration via integrin α9β1 (105). Integrin subunits α4 and α9 are both responsible for inhibition of cell spreading and enhanced cell migration (106,107). YIGSR, from Ln β1 chain, selectively binds the 67kDa laminin-binding receptor and promotes cell adhesion with no formation of focal adhesions or actin stress fibres (102,108). Finally, sequences from Fn C-terminal heparin-binding domain II such as WQPPRARI and SPPRRARVT bind cell surface proteoglycans syndecan-1 and syndecan-4, components of focal adhesions. Indeed, these immobilized peptides support cell adhesion with formation of focal adhesions and actin stress fibres (109).

4.3.2. Immobilization and cell responses

Adhesive signals. Ligand density and distribution on the surface influence cell behaviour. EC adhesion, spreading, focal adhesion formation and proliferation increase with RGD peptide or Fn surface density while EC migration is maximal for an intermediate concentration of RGD or Fn (91,96-98,101,110-112). Cell migration necessitates a turn-over of focal adhesions at the front edge. At low ligand density, cells cannot efficiently form new focal adhesions at the front while at high ligand density cells cannot break focal adhesions. RGD clustering, obtained by immobilization on a branched molecule, or increased peptide mobility in the layer, enhance integrin binding and cell response with minimum RGD densities, probably by favouring integrin clustering (99,113). EC polarization and directed migration is achieved on either RGD or Fn gradients or collagen or Fn stripes; migration speed increases by increasing concentration and decreasing stripes width, respectively (101,111,114,115). Finally, EC

immobilized on RGD surfaces preserve their differentiated phenotype and form a non-thrombogenic surface (102,116).

Combination with synergic peptides and protein fragments. As peptides only represent minimal binding sequences, they possess only a fraction of the activity of the entire protein and cells adhering on peptide surfaces usually show a decreased response that is probably related to non-optimized ligand conformation and/or absence of synergy sites reducing affinity for receptors. A solution is to co-immobilize the minimal RGD adhesive peptide with its synergy site PHSRN to increase adhesion. The PHSRN sequence found within the FnIII-9 domain of Fn does not support cell adhesion on its own but synergistically acts with RGD to increase α5β1 binding, cell adhesion and spreading (90,99,100). A peptide containing both the RGD cell binding and the PHSRN synergic sequences connected by a linker of appropriate length promote higher EC adhesion and spreading than mixed RGD and PHSRN (117). Equally, combination of the RGD peptide with syndecan-binding sequences from the Fn C-terminal heparin-binding domain II enhances EC proliferation and migration (109,118,119). EC response is even increased if cells adhere on peptides distributed as clusters on the surface (118). Another alternative is to immobilize a small Fn fragment containing the cell binding and synergy sites (120,121). Likewise, the REDV sequence from the CS5 domain of Fn does not support EC adhesion when incorporated in an artificial protein (122). However, incorporation of the entire CS5 region restores EC adhesion, probably by allowing the REDV binding sequence to adopt a recognizable conformation (123).

Another important objective in EC-material interaction studies is to be able to control EC proliferation and migration, which both require cell adhesion. To enhance cell migration, the RGD adhesive sequence can be co-immobilized with a pro-migratory peptide or fragment. Combination of RGD and YIGSR peptides lead to an increased EC migration (90). In addition, Fn N-terminal heparin-binding domain I interacts with α5β1, helping with the formation of Fn fibrils and promotes EC migration when immobilized (81). Hence, co-immobilized Fn cell-binding domain and heparin-binding I fragment enhance EC migration (2).

4.4. Growth factor immobilization

4.4.1. Cross-talk between integrins and growth factor receptors

Cell adhesion receptors such as integrins and growth factor receptors share a number of important signalling molecules, and the collaborative or mutual activation of integrins and growth factor receptors through their association results in signalling synergism and reciprocal potentiation (124,125). Once integrins and growth factor receptors are both bound to their specific ligands, they may cluster and act cooperatively. This results in an enhancement of growth factor-dependent responses (cell proliferation, motility or survival) when cells are attached to the appropriate matrix protein i.e., via the receptor-associated integrin. This type of collaboration has been observed for the VEGF receptor VEGF-R2, which physically associates with the αvβ3 integrin, inducing increased VEGF-R2 activation and EC proliferation when anchored to Vn, an αvβ3 ligand (126). Collaborative activation of integrins and growth factor receptors also results in increased expression and activation of integrins associated to collaborative receptors. Soluble VEGF enhances αvβ3 and α5β1 activation and subsequent EC adhesion and migration on Vn/osteopontin and Fn, respectively. Likewise, soluble FGF-2 promotes EC adhesion and migration on Vn via αvβ3 (72,127). In addition to collaborative activation, integrin engagement by a specific matrix protein can trigger ligand-independent activation of growth factor receptors and, on the other hand, growth factors can induce unligated integrins to propagate signals (124,125). Synergism of integrin and growth factor receptors signalling may be relevant in dynamic, multistep processes such as tissue development or regeneration, where cells undergo controlled migration and proliferation, and may differentiate. Hence, co-immobilization of integrin adhesive ligands and growth factors may provide a way to promote and achieve endothelialization of a biomaterial.

4.4.2. Surface-bound growth factors

Immobilized VEGF, a vascular specific growth factor, binds integrins αvβ3, α3β1 and α9β1 which mediate EC adhesion and migration (128,129). In combination with Fn, covalently immobilized VEGF enhances EC proliferation (70) and immobilization of a Fn fragment containing both α5β1 and VEGF binding domains promotes VEGF adsorption, increased EC migration and proliferation (89). Immobilized FGF-2 and hepatocyte growth factor (HGF) also promote strong and prolonged EC proliferation (130-132). However, surface-bound angiopoietins, regulators of angiogenesis, mediate cell adhesion, in part via integrins, but only Ang-1 could enhance EC spreading, focal adhesion formation and migration (133). A question remains whereas to covalently/firmly link growth factors to the surface or not. Soluble growth factors bind receptors on the apical side of the cell, they may form collaborative associations with integrins and they are internalized, which immediately cease the stimulated cell growth. On the other hand, adsorbed and covalently bound growth factors are exposed to the basal side, they can directly bind integrins (128-130,133) and, owing to their increased local concentration, immobilized growth factors may induce formation of integrin-growth factor receptor complexes that do not form in the presence of soluble growth factors (129,131), thus eliciting different intracellular signals. Moreover, firmly bound growth factors are probably not internalized, which explains their sustained activity. *In vivo*, blood vessel formation is a multistep process both locally and temporally controlled; interactions of cells with extracellular molecules, such as matrix proteins and growth factors, are usually transient. Non-permanent immobilization of growth factors may be required to achieve formation of a stable endothelium lining or mature functional blood vessels. It may be preferable to bind growth factors via natural interactions such as VEGF-Fn (89), to use recombinant growth factors with increased affinity for an ECM protein such as collagen type I (132),

or else, to chelate growth factors to a surface via surface-bound metal ions (134).

5. CONCLUSIONS AND PERSPECTIVES

Endothelial cells lining the luminal surface of blood vessels share common characteristics, they provide a non-thrombogenic surface and actively respond to stimuli from their microenvironment. However, they show great morphological and functional heterogeneity along the vascular tree depending on the vessel type and vascular bed. This diversity results from both local interactions with their microenvironment and epigenetic variations, mostly between macro- and micro-vascular endothelial cells (EC) (9,10). It should be mentioned that consequently, *in vitro*, EC originating from micro- or macro-vessels respond differently to a same stimulus (135). In addition, *in vivo*, EC constantly interact with matrix proteins which either maintain the endothelium integrity and functionality, or regulate, step by step, the formation of new blood vessels, by modulating response to soluble signals, during development or in physiological processes such as endometrium vascularization or wound healing.

Surfaces have been designed to mimic *in vivo* EC environment in a simplified way to promote EC ttachment, survival and phenotype preservation (non-thrombogenicity, angiogenic sprouting ability) for medical applications. In the past decades, EC have been exposed to various urfaces, pre-coated or not with adhesive matrix proteins or cell binding peptides, and results have shown that:

1. Material surface chemistry and the immobilization mode affect protein conformation, orientation and anchorage strength that in turn influence receptor affinity and selectivity as well as cell contractility and behaviour;
2. Peptide sequence and conformation enable targeting a specific receptor;
3. Cell-surface interactions can only be controlled if the ligand (s) is (are) immobilized on a low-fouling surface that prevents non-specific protein adsorption and thus “noise” signalling;
4. As adhesive peptides represent only the minimal binding sequence isolated from native proteins, co-immobilization with their synergic sequence or immobilization of a complete protein fragment enhances cell adhesion;
5. Integrin receptors collaboratively associate with other receptors, either other integrins or growth factor receptors, to coordinate and potentiate their signals. It should be advantageous to combine an adhesive signal such as RGD or Fn with a pro-migratory peptide/fragment or a growth factor to enhance EC migration and proliferation;
6. Directed cell migration can be achieved by physical or chemical contact guidance, immobilization of a gradient of adhesive molecules or cell exposition to fluid flow;
7.Formation of capillary-like structures on a surface depends on the cell-substrate adhesion strength, which relies on biochemical and mechanical signals.

The successful design of a biomaterial surface requires understanding and control of substrate physico-chemical properties, of protein-surface interactions, and of cell signalling *in vivo* and *in vitro*.

Endothelial cell retention on a surface correlates with strong binding to the surface and can be increased by shear stress pre-conditioning. Cell engineering by molecular biology techniques may be a way to artificially create cells with an enhanced potential to adhere on biomaterial surfaces. For instance, modified EC that co-express an adhesive matrix protein and VEGF better resist shear stress detachment (136). Likewise, biotinylated-EC incubated with streptavidin and exposed to surfaces presenting Fn and biotinylated-albumin, thus presenting a dual ligand system, show an increased retention when exposed to fluid flow (137). In addition to designing surfaces, molecular biology now enables researchers to engineer cells.

On the other hand, vascular in-growth, desired for implant integration or tissue engineering matrix vascularization, requires cell proliferation and migration. These cell processes should be tightly regulated, both spatially and temporally, to achieve the formation of a functional and stable endothelium. Local control can be obtained by surface patterning with matrix and growth factor cues, while temporal control may be accomplished by binding molecules to the substrate via linkers cleaved in response to cell secretion of proteases, for instance (5). Moreover, to acquire tissue specific vascular specialization or blood vessel stability, it would be interesting to co-culture EC with tissue cells or mural cells such as pericytes or SMC on patterned surfaces (8). Printing cell type specific domains would allow to simultaneous study and control cell-surface and heterotypic cell-cell interactions (138,139).

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Abbreviations: Ang: angiopoietin, EC: endothelial cell, ECM: extracellular matrix, FEP: fluoroethylenepropylene, FGF: fibroblast growth factor, Fn: fibronectin, HGF: hepatocyte growth factor, HS: heparan sulfate, HSPG: heparan sulfate proteoglycan, Ln: laminin, PETP: polyethyleneterephthalate (Dacron), PG: proteoglycan, PTFE: polytetrafluoroethylene, SMC: smooth muscle cell, TCPS: tissue culture polystyrene, TGF: transforming growth factor, Tsp: thrombospondin, VEGF: vascular endothelial growth factor, VEGF-R: VEGF receptor, Vn: vitronectin

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