Endothelial Function in Normal and Diseased Vessels

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C. Terracciano, S. Guymer (eds.), *Heart of the Matter*, Learning Materials in Biosciences,
https://doi.org/10.1007/978-3-030-24219-0_19

What You Will Learn in This Chapter

This chapter provides a comprehensive overview of the endothelium, its physiology and its dysfunction. We will begin by exploring the origin of endothelial cells and their importance in the regulation of blood pressure, inflammation and vascular homeostasis. From there, leukocyte transmigration will be expanded upon, with a discussion on relevant novel research. Next, the interplay of haemodynamic factors, namely differential flow patterns, on the endothelium will be explored, including several key mechanosignalling facets. Finally, the role of nitric oxide and endothelin-1 in regulating vascular tone will be examined.

Learning Objectives

- Outline the origin of endothelial cells and describe the key differences between their activated and natural quiescent states.
- Provide an overview of the main pathways and molecules involved in leukocyte transmigration.
- Outline the concept of shear stress, its role in endothelial homeostasis and the response of key mediators (nitric oxide and endothelin-1) in regulating vascular tone.

19.1 Origin of Endothelial Cells

Establishment of the vasculature is critical for embryonic development and postnatal life, allowing for the delivery of oxygen and nutrients to cells, in addition to the removal of waste products. Lining the vasculature are endothelial cells (ECs), which arise from mesodermal precursor cells (*haemangioblasts*) in the extraembryonic yolk sac and the embryo itself to form a de novo network of vessels in a process termed *vasculogenesis* [1]. Haemangioblasts form clusters known as blood islands, from which the outer layer gives rise to ECs. In their mature form, the endothelium comprises a single layer of flat cells that line the entire vascular and lymphatic systems [2]. These cells differ depending on their position within the cardiovascular system (CVS), with distinct transcription factor (TF) profiles allowing for the development of organ-specific ECs [1]. Broadly, the endothelium can be classified into three groups [2]:

- *Continuous*: ECs are tightly connected, with a continuous basement membrane. This is typical of the skin, muscle, lung and central nervous system (CNS).
- *Fenestrated*: the basement membrane contains areas of thinning where holes or fenestrae occur, classical of exocrine glands and the kidney.
- *Discontinuous*: marked gaps are present, with a poorly-formed underlying basement membrane allowing for high permeability. Found in the liver and bone marrow.

While different EC markers exist for different tissues, there is no universal marker present in all (and exclusively in) endothelial cells.

19.1.1 Resting Endothelium

The resting endothelium is quiescent and balanced towards an anti-inflammatory, anti-thrombotic, anti-apoptotic and anti-proliferative phenotype [3]. Activation of the endothelium disrupts this equilibrium in favour of a pro-inflammatory and pro-thrombotic state, the promoters of which include cytokines, low shear stress and smoking [3].

With ECs lining the entire circulation system, they play a significant role in cardiovascular health. Its aberrant, dysregulated activation, termed *endothelium dysfunction*, represents a critical step in the development of several cardiovascular diseases (CVD) including coronary artery disease, hypertension (HTN), stroke, peripheral vascular disease and venous thrombosis [3]. Collectively, these constitute a considerable global morbidity and mortality burden [3].

19.2 Endothelial Regulation

The endothelium plays a vital role in several aspects of physiology including angiogenesis, the local inflammatory response and haemostasis. Accordingly, dysfunction of this system is noted in the disease processes of several coagulation disorders (▶ Chap. 16) and almost all stages of atherosclerosis (▶ Chap. 17), to name a few.

19.2.1 Leukocyte Transmigration

Leukocyte transmigration arises during the local inflammatory response to trauma, infection, etc. and also pathophysiologically in atherosclerosis [4]. Leukocytes are recruited to the site to resolve the inflammatory stimulus and repair the surrounding tissue. Local chemokine releases from ECs and resident tissue macrophages, e.g., monocyte chemoattractant protein-1 (MCP-1), binds to receptors on inflammatory cells to promote their migration down a chemokine gradient [5]. Leukocytes undergo capture, rolling, firm adhesion and transmigration to enter tissues. These stages are summarised as follows.

19.2.1.1 Capture

Cell signalling proteins (cytokines) play a central regulatory role in inflammation, with two key mediators being tumour necrosis factor alpha (TNF-α) and interleukin-1 (IL-1). The presence of inflammatory cytokines upregulates the expression of P- and E-selectins by the endothelium, which bind to glycoproteins, e.g., P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes [6]. Of note, P-selectin is stored in EC granules and also in platelets, allowing immediate expression/release in response to pro-inflammatory stimuli [5]. This overexpression of selectins facilitates the formation of catch bonds, which capture leukocytes from the bloodstream, with the strength of the bond positively correlating with the force of blood [7]. This triggers a tyrosine kinase (TK)-mediated signalling cascade through the cytoplasmic tail, the outcome of which is an upright, high-affinity integrin conformation [5, 8]. Integrins are transmembrane proteins involved in intercell adhesion via their interactions with EC immunoglobulin ligands.

19.2.1.2 Rolling

The binding of PSGL-1 to selectins initiates a nexus of intracellular signalling pathways, e.g., the P38 MAPK pathway, which switch integrins into a high affinity state to facilitate their binding to ligands [9]. Two endothelial ligands of particular importance are [5, 10]:

- *Vascular Cell Adhesion Molecule-1* (*VCAM-1*): binds to 'very late antigen-4' (VLA-4) on monocytes.
- *Intercellular Adhesion Molecule-1* (*ICAM-1*): binds to 'lymphocyte function-associated antigen-1' (LFA-1) on monocytes.

Both of these adhesion molecules facilitate leukocyte rolling and are upregulated by inflammatory cytokines, especially TNF-α. There is stronger binding between leukocytes and the endothelium through this change in integrin conformation. This process is termed *inside-out signalling* [8].

19.2.1.3 Slow Rolling

Bond strength is then increased via *outside-in signalling*, whereby receptors cluster together to increase valency and the formation of stronger bonds with leukocytes via the recruitment of additional tyrosine kinase and PI3K (phosphoinositide 3-kinase) signalling [8, 11]. Moreover, outside-in signalling also regulates the subsequent steps of leukocyte transmigration.

19.2.1.4 Firm Adhesion

The eventual outcome of chemokine-mediated slow rolling is leukocyte arrest, assisted by a potpourri of leukocyte surface integrins [5]. A key interaction is between LFA-1 integrins and ICAM-3 on the endothelium, which results in Ca^{2+} mobilisation, adhesion and the secretion of further chemokines [12]. Finally, immunoglobulin-like domains on leukocyte surface ligands form strong disulphide (covalent) bonds with the endothelium.

19.2.1.5 Leukocyte Transmigration

The transmigration of leukocytes out of the vasculature relies on both 'docking structures' found on ECs that are rich in ICAM-1 and VCAM-1 [13], and a host of endothelial cell-cell junction molecules, three of which are [14, 15]:

- *Junctional Adhesion Molecules* (*JAMs*): bind to integrins to propagate the movement of leukocytes through intercellular spaces. They are constitutively expressed at endothelial junctions and can be induced on the endothelial surface following inflammatory stimuli.
- *Platelet Endothelial Cell Adhesion Molecule-1*(*PECAM-1*): interact with cytosolic catenins to facilitate migration.
- *Vascular Endothelial (VE)-Cadherin*: an adhesion molecule whose expression on opposing endothelial surfaces normally contributes to the formation of tight intercellular junctions via homophilic interactions [16]. This maintains the structure of the endothelium.

VE-cadherin is constitutively phosphorylated and then dephosphorylated. However, loss of phosphatase activity through leukocyte binding leads to VE-cadherin phosphorylation and resultant EC dissociation. This disruption of the endothelial integrity increases permeability to promote leukocyte transmigration [16].

Transmigration is either *paracellular* (in the intercellular space between cells) or *transcellular* (through a cell) [5]. Paracellular transmigration involves cells passing between cells without increasing permeability, possibly via the induction of Rho-GTPases by ICAMs, or by increased intracellular Ca^{2+} that binds to myosin light chain kinase to trigger EC contractions. In contrast, transcellular transmigration is thought to occur via the extension of leukocyte projections into ECs to trigger a complex cytoplasmic signalling system that facilitates vesicle organelle channels, through which the leukocyte can migrate. This entire process is summarised in Fig. 19.1.

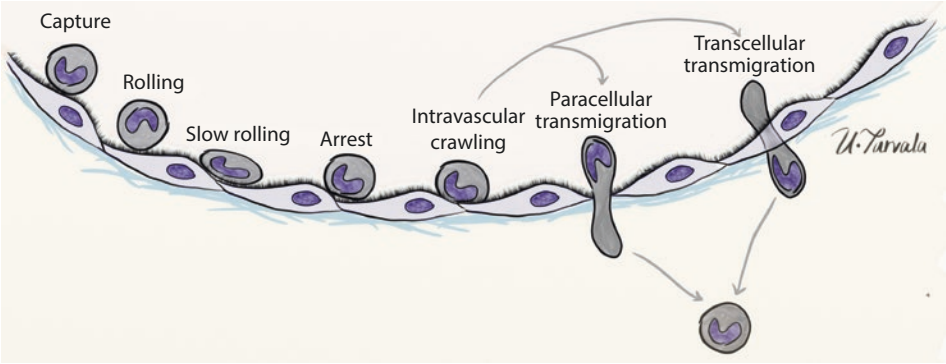

Fig. 19.1 Diagram summarising the canonical five-stage process of leukocyte transmigration, namely capture, rolling, slow rolling, adhesion and transmigration. Key interactions are shown, including VCAM-1/VLA-4 and ICAM-1/LFA-1

19.3 Mechanotransduction and Mechanosignalling

Shear stress is the frictional force per unit area that endothelial cells are constantly exposed to by blood flow [17]. *Linear laminar pulsatile flow* refers to the rapid, high shear stress profile of flow that runs parallel to the endothelium and activates several cytoprotective pathways.

The resulting signalling is anti-thrombotic, anti-adhesive, anti-proliferative, anti-inflammatory and anti-apoptotic and induces the synthesis of nitric oxide (NO), prostacyclin and tissue plasminogen activator (tPA) [18, 19]. Typically, soluble molecules released by endothelial cells in response to laminar flow help in maintaining a stable quiescent vessel wall. However, exceedingly high shear stress can injure and erode the endothelium, causing it to become prothrombotic [20]. In contrast, low shear stress occurs at the inner curvatures of vessels, whereby bifurcations and branch points cause disturbed blood flow, slowing its velocity and creating non-linear and turbulent patterns [18]. This oscillatory flow is pro-inflammatory and associated with plaque formation [19].

The entire process of mechanosensing and mechanotransduction in endothelial cells is termed *mechanosignalling* and can result in either the maintenance of vascular homeostasis or contribute to endothelial cell dysfunction and atherosclerosis.

19.3.1 Mechanosensors

ECs detect patterns of flow, and thus variation in shear stress, using a multitude of receptor types expressed on their surface. Let us explore some of the identified mechanosensors capable of detecting alterations in mechanical input/flow:

Glycocalyx a glycolipid and glycoprotein complex that coats the inner endothelial surface. Alteration or removal of one of its constituents, heparan sulphate, impairs flow-mediated responses, including the induction of flow-mediated genes [21]. One heparan sulphate proteoglycan, syndecan-4, has previously been characterised as a critical mechanosensor whose loss is associated with suppression of KLF2/4 signalling and an increased susceptibility to atherosclerosis [21, 22].

Caveolae small invaginations of the plasma membrane, with caveolin (cav)-1 required for the EC response to shear stress via shear-induced ERK activation [23].

Primary Cilia dysfunction is associated with atherosclerotic plaque sites in regions of disturbed flow. Non-ciliated cells show a decrease in flow response, with less induction of KLF2 [24].

Ion Channels calcium, sodium, potassium and chloride shear-sensitive ion channels are the fastest known response to shear stress [25]. Deletion of specific channels can lead to the loss of shear-induced vasodilation, NO production and atheroprotective signalling [26].

G Protein-Coupled Receptors conformational changes occur to receptors such as those for bradykinin when exposed to shear stress [27]. Bradykinin agonists inhibit shear-induced mechanisms.

EC Nuclei the nuclei of endothelial cells have been shown detect both the strength and direction of blood flow, ultimately leading to transcriptional and phenotypic changes in the EC [28].

Integrins shear stress induces the rapid activation of select integrins. Blocking integrin signalling prevents the activation of shear-sensitive signalling pathways [29].

Junctional Proteins PECAM-1, VE-cadherin and VEGFR2 (vascular endothelial growth factor receptor 2) are all required to form a shear-responsive mechanosensory complex [30].

19.3.2 Mechanosignalling

Following induction by mechanosensors, several pathways comprise the mechanosignalling response. Some of these include:

Krüppel-Like Factor 2 (KLF2) the first transcription factor identified to be shear sensitive. The interactions between KLF2 and KLF4 play a role in mediating the anti-inflammatory, atheroprotective effects of laminar flow [31].

Senescence disturbance of flow encourages senescence (growth arrest) of endothelial cells via the p53-p21 pathway. This can be inhibited through the histone deacetylase enzyme sirtuin 1, which is activated by restoration of normal flow patterns; it is also activated pharmacologically [32]. Interestingly, resveratrol in some foods and wine also promotes sirtuin 1 expression, reducing the senescence of ECs and thus protecting them [32].

CD59 inhibits the terminal complement pathway by preventing the formation of membrane attack complexes (MAC). Kinderlerer et al. observed upregulated CD59 expression in the presence of laminar shear flow, yet this was dependent upon other key regulators, e.g., KLF2 expression [33]. Disturbed flow did not show any significant induction of CD59 expression. Hence, laminar shear stress can protect against complement-mediated injury by increasing CD59 levels [33].

19.4 What We Don't Know: Sirtuin 3 in Mechanosignalling

Sirtuin 3 (SIRT3) is a mitochondrial enzyme with several key roles specific to cardioprotection, sprouting angiogenesis, EC metabolism and EC-cardiomyocyte interactions [34]. It is thought that in some circumstance, endothelial cells in the coronary vasculature primarily utilise glycolytic metabolism to save oxygen for the adjacent cardiomyocytes located in the heart [34]; Interestingly however, He et al. identified SIRT3-deficient ECs as exhibiting decreased rates of glycolysis and increased mitochondrial oxygen consumption [35]. This may limit available oxygen for cardiomyocytes, potentially resulting in cardiomyocyte dysfunction and necrosis.

Overexpression of SIRT3 improves insulin resistance in ECs, whereas downregulation, using small interfering RNA (siRNA), proved to increase mitochondrial ROS and reduce the insulin response in human umbilical vein ECs [36]. Endothelial-dependent vasorelaxation also diminishes SIRT3 deficiency that is found in obese human and mice subjects [36].

19.5 Vascular Tone

We have already discussed in previous chapters how the interplay of several different systems collectively regulates vascular tone, and the instrumental role the endothelium has. Accordingly, the importance of nitric oxide (NO) as a vasoactive regulator has been explored, and how its downregulated expression is a salient feature of early endothelial dysfunction. A thorough understanding of NO is vital to appreciate the critical role it has in modulating vascular tone.

19.5.1 Nitric Oxide

NO is synthesised by endothelial nitric oxide synthase (eNOS) normally bound to caveolin proteins located in cell membrane invaginations called *caveolae* [37]. Increased intracellular Ca^{2+} (of both extracellular and endoplasmic reticulum origin) increases the probability of Ca^{2+} binding to calmodulin. This causes a structural change in calmodulin, facilitating its binding to eNOS, causing eNOS to simultaneously detach from caveolin and activate [37]. In the presence of specific cofactors, e.g., tetrahydrobiopterin, activated eNOS then catalyses the synthesis of NO from L-arginine [37].

NO produces protective anti-thrombotic, anti-adhesive and anti-proliferative effects on the vasculature. Because of this, the dysregulation of NO represents an essential stage of endothelial dysfunction [38]. Briefly, this occurs when eNOS switches from generating NO to producing superoxides and hydrogen peroxide, a process termed *eNOS uncoupling* [38]. eNOS uncoupling occurs in areas where there is local excess of reactive oxygen species (ROS) that leads to the uncoupling of tetrahydrobiopterin from eNOS, with subsequent diminishment of nitric oxide biosynthesis [39].

Physiologically, NO diffuses from the endothelium into VSMCs, where it binds to and activates guanylyl cyclase, catalysing the conversion of GTP to cyclic GMP (cGMP) [37]. This acts to decrease smooth muscle tension via a reduction in Ca^{2+} release from the SR. This pathway has been targeted therapeutically, with the development of nitroglycerin

(prodrug) and sildenafil (a phosphodiesterase-5 inhibitor that prevents cGMP degradation). Of note, laminar shear stress increases AKT-mediated phosphorylation of eNOS, enhancing its catalytic activity [40].

19.5.2 Endothelin-1

Another important modulator of vascular tone is endothelin-1 (ET-1): a peptide synthesised and secreted by ECs, which mediates some of its effects on these cells directly [41]. It contributes to both vascular tone and the regulation of cell proliferation through its binding to ETA and ETB receptors, present on cells of the CVS, namely cardiomyocytes, fibroblasts, smooth muscle and ECs, and also tubular and glomerular cells in the kidney [41]. Though previously considered purely as a vasoconstrictor, the current consensus is that ET-1 has an effect on vascular remodelling, angiogenesis and extracellular matrix synthesis.

- ET-1 acts at endothelial ETB receptors, inducing the production of NO and prostacyclin to mediate vasodilation and angiogenesis [41].
- ET-1 acts at ETA receptors on VSMCs to bring about vasoconstriction and proliferation. This is through Ca^{2+} acting as a second messenger, resulting in SR Ca^{2+} channel opening and subsequent vasoconstriction [41].
- ET-1 signalling activates macrophages, elevating the production of free radicals [41].

19.6 Clinical Implications: Replacement of Damaged Endothelium

Endothelial dysfunction and damage result in the loss of endothelial integrity. Consequently, endothelial cells detach and enter the circulation, allowing their utilisation as a marker of damage [42]. Replacement of the damaged endothelium occurs by either division of the surrounding ECs or by the actions of endothelial progenitor cells.

Cardiovascular risk factors, e.g., smoking, impairs the mobilisation of these endothelial progenitor cells, as well as their differentiation and function [43]. However, it has been demonstrated that increased numbers of these circulating ECs can offset the impairment resulting from cardiovascular risk factors [43]. Furthermore, exercise and statins have been shown to have a beneficial effect on the mobilisation of these progenitor cells [44, 45].

Take-Home Message

- Endothelial cells line the entire vasculature in a quiescent state unless activated to a pro-inflammatory, prothrombotic state. This change in phenotype contributes to a range of cardiovascular diseases that impose a significant health burden.
- Leukocyte transmigration mediates their recruitment to areas of local inflammation to either aid defence systems or as part of a wider inflammatory pathophysiology.
- Laminar/high shear flow exerts a cytoprotective effect on the endothelium, whilst disturbed/low shear flow is pro-inflammatory.
- Endothelial dysfunction is characterised by an imbalance between vasodilatory and vasoconstrictive molecules including nitric oxide, prostacyclin, bradykinin and endothelin.

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