

Cytoskeleton, cytoskeletal interactions, and vascular endothelial function

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Abstract: Far from being inert, the vascular endothelium is a critical regulator of vascular function. While the endothelium participates in autocrine, paracrine, and endocrine signaling, it also transduces mechanical signals from the cell surface involving key cell structural elements. In this review, we discuss the structure of the vascular endothelium and its relationship to traditional cardiovascular risk factors and clinical cardiovascular events. Further, we review the emerging evidence that cell structural elements, including the glycocalyx, intercellular junctions, and cytoskeleton elements, help the endothelium to communicate with its environment to regulate vascular function, including vessel permeability and signal transduction via nitric oxide bioavailability. Further work is necessary to better delineate the regulatory relationships between known key regulators of vascular function and endothelial cell structural elements.

Keywords: endothelium, shear stress, eNOS, cardiovascular risk factors, glycocalyx

Introduction to the vascular endothelial cell

The monolayer of cells lining the innermost surface of the circulation system including the heart chambers and blood vessels was originally named the “endothelium” by Swiss anatomist Wilhelm His Sr in 1865.^{1,2} Since then, endothelial cells have been extensively studied.^{3–13} Early hypotheses that the endothelium was a static, passive mechanical barrier, present solely to separate the blood flow from the vessel wall and tissues, have given way to the modern concept of the endothelium as a key, biologically active, cell layer.

In the average adult, the endothelium has a net weight of ~1 kg and lines a total surface area measuring 300–1000 m².^{1,6,14} Endothelial cells are primarily mononuclear cells with slightly basophilic cytoplasm containing various intracellular organelles (eg, the Golgi complex, mitochondria, endoplasmic reticulum, and Weibel–Palade bodies).^{1,15,16} Endothelial cells can become multinucleated under both normal¹ and atherosclerotic conditions,^{17,18} although the significance of this phenomenon is unclear. The average endothelial cell measures approximately 50 × 11 μm (length × width),¹ with a thickness ranging from 0.1 to 1.0 μm.^{1,16,19} However, the height of endothelial cells vary relative to their exposure to shear and anatomical location.²⁰

Endothelial cells are generally polygonal²¹ and appear similar to cobblestones.^{22,23} Their shape is determined by multiple factors, including the cell structural elements, cell metabolism, mechanical forces, and cell–cell and cell–matrix interactions.^{1,24} For example, the arterial endothelium lining tends to be thinner and more parallel to the direction of blood flow than the venous endothelium. Younger endothelial cells with

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faster growth rates are thicker than older cells.¹ Shape also changes with vascular tone.

Roles of the vascular endothelium

Regulation of vasodilation and angiogenesis

The non-diseased endothelium regulates pro-vasodilatory and vasoconstrictive influences, balancing autocrine, paracrine, and endocrine influences to maintain normal vascular physiology. Nobel Prize-winning work revealed that a central paracrine factor responsible for regulating vascular function is nitric oxide (NO).^{25–27} NO is synthesized in the endothelium by nitric oxide synthase (eNOS) through the enzymatic conversion of L-arginine to L-citrulline. After diffusing into smooth muscle cells, NO generates cyclic guanosine monophosphate from guanosine triphosphate by activating soluble guanylyl cyclase. Further, NO induces vascular smooth muscle relaxation by decreasing cytosolic Ca²⁺ and dephosphorylating myosin light chains in smooth muscle cells.^{28–30} NO can also directly activate the big conductance calcium-activated potassium channel (BKCa) channel on the membrane of smooth muscle cells to cause vasodilation.¹⁶ Other vasoactive factors produced by the endothelium include endothelial-derived hyperpolarizing factors such as hydrogen peroxide, cytochrome 450 metabolites, C-natriuretic peptide, prostacyclin, and endothelin-1.^{6,16,25,31} Endothelial cells also secrete various growth factors, including vascular endothelial growth factor, to participate in endothelial regeneration and angiogenesis.^{32,33}

Control of vascular inflammation processes

The endothelium is essentially devoid of evidence of inflammation under homeostatic conditions.³⁴ However, the endothelium plays a central role in integrating local and systemic inflammatory signals, leading to alterations in the endothelial phenotype characterized by increased local and systemic endothelial inflammation.^{35–37} Interestingly, NO bioavailability plays a central role in regulating the inflammatory response. Normal homeostatic levels of NO limit endothelial expression of adhesion molecules (vascular cell adhesion protein 1, intercellular adhesion molecule 1, and P-selectin)^{16,38} and the production of leukocyte/monocyte recruitment factors.^{39,40} This prevents the adherence of leukocytes and monocytes from the bloodstream onto the endothelial surface.^{41–43} Further, NO helps to maintain

normal endothelial layer permeability by stabilizing physiological cytoskeleton distribution, inhibiting penetration of pro-inflammatory cells into subendothelial space.⁴⁴ Reduced NO bioavailability may disrupt the cytoskeleton-dependent structural integrity of the endothelium by inhibiting Rho activity.⁴⁵

Regulation of thrombosis and fibrinolysis

The normal vascular endothelium also carefully balances prothrombotic and pro-fibrinolytic factors.⁴⁶ Endothelium-derived NO plays a central role in this balance by limiting platelet activation and adhesion.⁴⁷ NO inhibits platelet aggregation by inhibiting key platelet–platelet interactions.^{48,49} NO also inhibits the expression of plasminogen activator inhibitor-1, stimulating fibrinolytic pathways.⁵⁰ Factors other than NO play complimentary roles in regulating thrombosis and fibrinolysis at the level of the endothelium. For example, thrombomodulin expressed on endothelial cells binds thrombin to block thrombin-triggered thrombus formation. Further, thrombomodulin reduces activated factor V through activation of proteins C and S to inhibit thrombin formation.⁵¹

Endothelial dysfunction and cardiovascular risk

Traditional (hypertension, dyslipidemia, aging, diabetes mellitus, smoking) and emerging (eg, inflammation, hyperhomocysteinemia) cardiovascular risk factors impair endothelial function in humans at least in part through reduced NO bioavailability.⁴⁶ The inflamed, prothrombotic, pro-proliferative, and vasoconstrictive phenotype observed secondarily to reduce NO bioavailability is commonly referred to as “endothelial dysfunction.”

Individual risk factors induce a dysfunctional endothelial phenotype through multiple mechanisms. While an exhaustive description of the effects of each cardiovascular risk factor on the vascular endothelium are beyond the scope of this review, a brief discussion of the effects of key traditional risk factors on endothelial function will give a sense of the common effects of otherwise disparate risk factors on the vascular endothelium. Hypertension induces vascular inflammation and excessive reactive oxygen species (ROS) production from multiple sources, including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and uncoupled eNOS.^{52,53} Elevated angiotensin II levels also contribute to hypertension, at least in part through increased NADPH oxidase activity.^{54–57}

Endothelial function is impaired and blood pressure is increased in eNOS synthase knockout animals,⁵⁸ further indicating that eNOS is a key factor in maintaining normal vascular function.

The insulin resistance also impairs endothelial function through reduced NO bioavailability and increased oxidative stress.^{59,60} Hyperglycemia exposure increases endothelin-1 secretion⁶¹ and impairs NO bioavailability, both through reduced eNOS activity and increased NO destruction. Further, hyperglycemia in insulin resistance also increases vascular oxidative stress through uncoupling eNOS,^{62–64} activating NADPH oxidase,⁶⁵ inhibiting ROS scavenging systems,⁶⁶ and stimulating mitochondrial ROS production.^{67–69}

Hypercholesterolemia also impairs endothelial function.^{70–75} Oxidized low-density lipoproteins (oxLDL) disrupt NO production from eNOS function by reducing the bioavailability of tetrahydrobiopterin (BH4), a key co-factor for NO production from eNOS. Loss of BH4 bioavailability^{76–79} encourages superoxide production from eNOS rather than NO. Administration of BH4 to patients with hyperlipidemia blunts hypercholesterolemia-associated endothelial dysfunction.^{80–82} oxLDL upregulates the production of the endogenous eNOS inhibitor, NG-NG-dimethyl-L-arginine (asymmetric dimethylarginine)^{83,84} and promotes the association of eNOS with caveolin-1.⁸⁵ In addition, oxLDL elicits a vascular inflammatory response and increases endothelial permeability to inflammatory cells, inducing a vicious cycle leading to the formation of atherosclerotic plaques.^{86–88}

In summary, the vascular endothelium integrates the effects of extrinsic influences on the vascular endothelium with intrinsic susceptibility to external influences on endothelial health, making measurements of endothelial function an excellent “barometer” of overall cardiovascular risk (eg, the likelihood of cardiac angina, myocardial infarction, stroke, or peripheral vascular disease).⁸⁹ Multiple studies have demonstrated that the presence of endothelial dysfunction in large conduit vessels, coronary arteries, and resistance arterioles predicts future cardiovascular events in humans both with and without prevalent coronary artery disease.^{46,90} Pharmacological interventions targeting cardiovascular risk factors are more efficacious when they concomitantly reduce cardiovascular risk.^{91,92}

While the clinical application of measurements of endothelial function remains limited,^{46,90} these data strongly support the concept that readily measureable endothelial

function provides key information on overall cardiovascular health. In the following sections, we will discuss the role of endothelial structural elements that influence overall endothelial function.

Relationships between endothelial structural elements and endothelial function

Glycocalyx

The endothelium is in direct contact with the blood's fluid; cellular components (erythrocytes, leukocytes, and platelets); macromolecules such as lipids; proteins and carbohydrates; and small molecules such as water, glucose, and ions. Under normal homeostatic conditions, blood components that stimulate vascular inflammation, injury, and thrombus formation generally do not adhere to endothelial cells. This biological feature is, at least in part, attributable to an anti-inflammatory and anti-thrombogenic surface on the endothelium, the glycocalyx.⁹³ The glycocalyx is a uniform, semipermeable layer (on average ~50 to 100 nm thick) composed of proteins, glycoproteins, glycolipids, and proteoglycans.¹² The net electrostatic charge of the glycocalyx is negative at physiological pH levels^{93–95} due to heparin-like sulfated mucopolysaccharides and neuraminidase-sensitive (or -resistant) anionic groups within the layer.^{95–97} Because of electrostatic repulsion, negatively charged erythrocytes, leukocytes, and low-density lipoprotein (LDL) cholesterol are repelled from the surface of the endothelium, inhibiting attachment to the endothelium.^{94,98} This repulsion results in an erythrocyte-free plasma layer along the endothelium, decreasing hemoglobin-scavenging of NO and preserving overall endothelial NO bioavailability.⁹⁹

The glycocalyx is organized by core proteins in the proteoglycan clusters and these proteins attach to the intracellular cortical cytoskeleton bridged to integrin-containing focal adhesion molecules in the extracellular matrix by alpha actinin and actin stress fibers.⁹⁸ The structural relationship between the glycocalyx and the cellular cytoskeleton appears to require co-localization of integrin with syndecan-4.^{100,101} Syndecan-4, a trans-membrane protein, connects with the cytoskeleton by its cytoplasmic tail and participates in cytoskeleton organization via mechanotransduction.¹⁰²

The mesh network of the glycocalyx works as a molecular sieve to adsorb plasma protein molecules such as albumin, fibronectin, and fibrinogen. This helps to establish an elastic interface that can withstand the mechanical forces to which it is subjected to by pulsatile blood flow.^{12,93,103} The hydraulic

resistance of the glycocalyx and its matrix is approximately 108–1011 dyn s/cm⁴.¹²

Many pathological factors, such as oxLDL, salt-sensitive hypertension, and diabetes have been shown to be able to disturb the anionic charge, structure, and distribution of the glycocalyx.^{95,104,105} This leads to increased cell permeability and recruitment of pro-inflammatory and pro-atherogenic factors.⁹⁵ Excessive oxidative stress mechanistically contributes to hypoxia and hyperlipidemia disrupts the glycocalyx.^{95,106} Further, reduced heparin sulfate in the glycocalyx, secondary to excessive oxidative stress, reduces transport of L-arginine to the endothelial surface, leading to a decrease in NO production.

Cell–cell junctions

While our appreciation of the potential role alterations in the glycocalyx may play in the development of endothelial dysfunction continues to grow, other extracellular structures – in particular, cell–cell junctions – also play an important role in regulating endothelial function. Cell–cell junctions are critical to the maintenance of normal endothelial cell barrier functions and mediate intercellular signaling conduction. The three types of junctions between endothelial cells include tight, gap, and adherence. The protein components of tight junctions are zonula occludens 1, cingulin, and a small guanosine triphosphate-binding protein, rab 13. These molecules mediate intracellular signal transduction by anchoring tight junctions to actin microfilaments.³ Gap junctions are composed of connexins (Cx37, Cx40, Cx43), while adherence junctions consist of cadherins and cytoskeletal proteins α -catenin and β -catenin vinculin, and plakoglobin.^{9,107} Tight junctions seal off paracellular clefts to limit intercellular permeability. Phosphorylation of zonula occludens 1 increases vascular permeability, a mechanism involved in diabetic vasculopathy.^{9,108} The primary role of gap junctions is to mediate electrochemical signaling via low-resistance conduction pathways – these signals govern cell migration and replication.^{3,19,109,110} Gap junctions may play an important role in endothelium-dependent vasodilation and blood pressure regulation.^{111,112} Reduced connexin levels in gap junctions are found in hypertensive and diabetic animals with phenotypical endothelial dysfunction.^{112,113} In adherence junctions, cadherin connects with catenin via its cytoplasmic domain, forming a cadherin-catenin complex. These complexes anchor to actin microfilaments of the cytoskeleton to mediate cell–cell interaction.^{3,9}

In addition to the three types of junctions delineated above, platelet-endothelial adhesion molecule 1 (PECAM-1),

an endothelial adhesive molecule belonging to the immunoglobulin family, also participates in endothelial cell–cell recognition, cell junction assembly, and leukocyte extravasation.^{3,9} PECAM-1 participates in modulating NO signaling by mediating shear stress-induced phosphorylation of eNOS and Akt enzymes in endothelial cells.¹¹⁴ Another endothelial adhesive molecule, integrin, helps anchor endothelial cells onto the extracellular matrix, playing a critical role in maintaining endothelial cell permeability.^{3,115} Integrins also mediate shear-induced signaling transduction via the Src/extracellular signal-regulated kinase/phosphatidylinositol 3-kinase pathway or by inducing PECAM-1 tyrosine phosphorylation.¹¹⁶

Cytoskeleton

The endothelium is subjected to multiple mechanical forces, including laminar shear stress, cyclic strain, hydrostatic pressure, and pulsatile pressure.¹¹⁷ To maintain structural integrity, the endothelium must be resilient enough to withstand these stressors. In concert with the glycocalyx and intercellular junctions,^{12,93} the endothelial cell cytoskeleton plays a central role in maintaining cellular structural integrity and transducing cellular responses to mechanical forces.^{9,94,95,98,118,119}

The endothelial cytoskeleton is composed of three types of filaments: microfilaments, microtubules, and intermediate filaments.¹¹⁸ Of these, microfilaments and microtubules appear to be the most important in regulating endothelial cell structure and function. Microfilaments (200–500 nm diameter) are comprised of a contractile protein, actin, either in monomeric form (globular or G-actin) or filamentous form (F-actin). Actin microfilaments form dense peripheral bands in the cell periphery and stress fibers in the central portions of endothelial cells.¹¹⁸ Actin microfilaments can change their orientation, distribution, and alignment based on the type of shear exposure. For example, in high laminar shear states, actin forms more prominent central bands than under low laminar shear states.¹¹⁸ Under turbulent flow, cytoskeletal remodeling promotes the atherosclerotic processes by altering the endothelial architecture and molecular responses toward favoring endothelial dysfunction.¹¹⁷ Vascular inflammation is associated with endothelial cytoskeletal redistribution leading to increased intercellular gap size and paracellular permeability.¹¹⁸ Actin fibers attach to integrins on the cytoplasmic side of the cell surface, allowing microfilaments to participate in integrin-mediated signaling that may result in cell–cell adhesion, cell differentiation, and cell proliferation.^{45,120} Microtubules are formed by tubulin

heterodimers of α and β subunits¹²¹ and are distributed throughout the cytoplasm. Microtubules play a major role in the transportation of intracellular vesicles and localization of membrane-bound organelles. Functioning microtubules are essential for maintaining shear-induced NO production¹²² and vasodilation.¹²³

Common cardiovascular risk factors known to reduce NO bioavailability, including hyperlipidemia, hypertension, diabetes, and smoking,⁴⁶ alter microfilament structure and distribution.^{124–127} NO inhibition changes the distribution of actin filaments in endothelial cells, while shear alters actin orientation in an NO-dependent manner.^{44,128} As a major component of ribonucleoprotein binding with eNOS mRNA untranslated region, G-actin increases eNOS mRNA instability. Therefore, lower G-/F-actin ratios are associated with a higher eNOS expression.¹²⁹ This regulation of eNOS expression by actin may be clinically important in limiting neurological damage from strokes.¹³⁰ Endothelial nuclear β -actin appears to upregulate eNOS expression by binding onto the 27nt repeat element of eNOS, intron 4,¹³¹ which suppresses eNOS expression via histone acetylation and DNA methylation.¹³²

Intact microtubules are important in maintaining eNOS activity in pulmonary vascular endothelial cells¹³³ and NO-dependent vasodilation in other key arterial beds.¹³⁴ Microtubules may exert their regulatory influence on eNOS by altering cellular distribution of proteins and co-factors directly related to NO production, including arginase.¹²² oxLDL may increase arginase activity by disrupting microtubules, which may in part account for reduced NO bioavailability in hypercholesterolemia.¹³⁵ Angiotensin II, commonly elevated in hypertension and endothelial dysfunction,¹³⁶ induces microtubule disassembly.¹³⁷ Adiponectin, an adipocyte chemokine that is suppressed in obesity, protects the endothelium against inflammatory stimuli partly by reducing microtubule disassembly, as well as through protecting gap junctions and actin fibers.¹³⁸ Taken together, these data strongly suggest microtubule disassembly, induced by common cardiovascular risk factors, is an important event in the development of endothelial dysfunction.

Involvement of cytoskeletal elements in flow-induced endothelial cell signaling

As discussed previously, the endothelial cytoskeleton is deeply interconnected with the cell surface, cell junctions, and focal adhesion molecules. An intact cytoskeleton appears to

be central to flow-induced signaling, particularly the eNOS-dependent NO production vital to endothelium-dependent vasodilation in multiple vascular beds. Experimental disruption of microtubule structure in isolated rat arterioles leads to an impaired flow-mediated dilation.¹²³ Inhibition of both actin filaments and microtubules interferes with vasodilatory signaling in human coronary arterioles and the pulmonary circulation,^{139–141} suggesting the importance of the integrity of endothelial cytoskeleton in regulating endothelial-dependent vasodilator signaling transduction.

Multiple mechanisms are responsible for the intrinsic connection between cytoskeletal elements and NO-dependent vasodilation. First, shear-induced increases in intracellular calcium require intact actin microfilaments.^{107,142} An intact cytoskeleton may also be important in shear-induced, Akt-dependent phosphorylation/activation of eNOS.^{143,144} Laminar shear increases NO production by increasing eNOS-heat

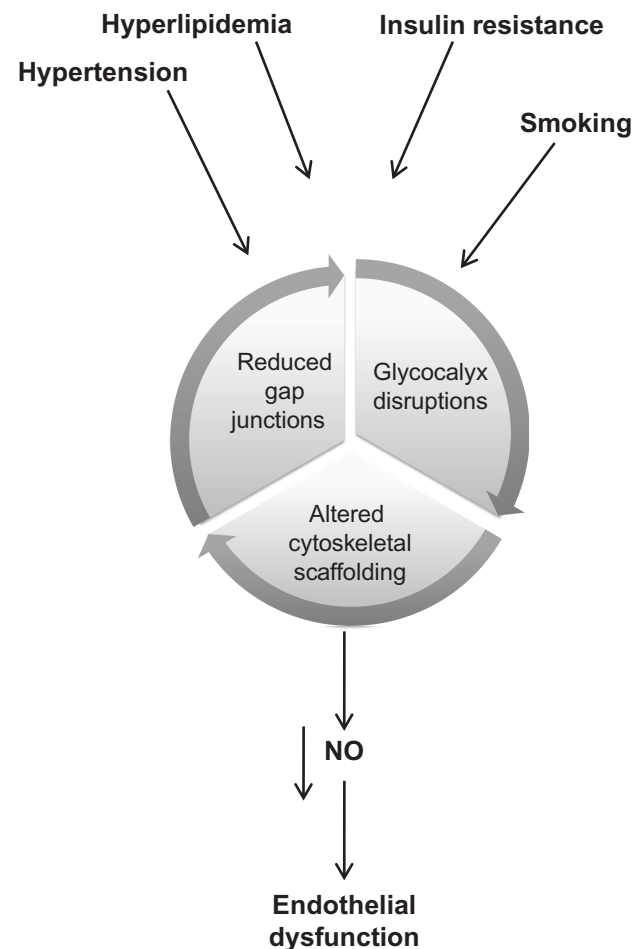


Figure 1 Traditional cardiovascular risk factors induce alterations in endothelial cell structure associated with impaired nitric oxide (NO) bioavailability and subsequent endothelial dysfunction.

Notes: Traditional cardiovascular risk factors alter the endothelial cell surface, cell–cell adhesion molecules, and the cytoskeleton, which appear mechanistically important in suppressing NO bioavailability and subsequent endothelial dysfunction.

shock protein⁹⁰ interactions required for NO production. This interaction is stabilized by both tubulin and actin microfilaments.¹⁴³ Shear stress-induced reorganization of the cytoskeleton may influence eNOS activity and NO production by regulating eNOS–caveolin-1–actin filament associations.¹⁴⁵

Downstream transmission of shear signaling appears to be mechanistically linked to modulation of mitochondrial ROS production and the motion of the nuclear karyoskeleton.¹⁰⁷ Actin transmits shear stretch signals to mitochondria, triggering the release of mitochondrial ROS production, activation of nuclear factor kappa B,^{140,146} and reduction of NO bioavailability that occur concomitantly with mitochondrial inner membrane hyperpolarization.¹⁴⁷ Through interaction with p47phox, the actin cytoskeleton in endothelial cells also plays a role in regulating the NADPH oxidase-derived ROS level during shear-induced reorganization.¹⁴⁸ Taken together with our knowledge of the mechanistic links between NO and cytoskeletal elements, the concept of coordinate regulation between cytoskeletal elements and traditional endothelial cell signaling cascades responsible for endothelial function is well supported.

Conclusion

Overall, the current data support the concept that the endothelium's "form" mirrors its "function." Traditional cardiovascular risk factors known to affect vascular signaling leading to endothelial dysfunction also have characteristic effects on endothelial cell structure (Figure 1). Further, vascular endothelial cell signaling, particularly in relation to shear, appears intrinsically related to the endothelial cell surface, cell–cell interactions, and cytoskeletal elements. These data also suggest that the endothelial cell structural components could be potential targets for pharmacological therapies to improve vascular homeostasis. Significant work remains to be done to unravel how key vascular regulators such NO are mechanistically linked to cytoskeletal and other cell structural components and this information can be best used to improve cardiovascular health.

Disclosure

The authors report no conflicts of interest in this work.

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