Junctions at the Crossroads: The Impact of Mechanical Cues on Endothelial Cell-Cell Junction Conformations and Vascular Permeability

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Abbreviations: VE-cadherin, vascular endothelial-cadherin; ZO, zonula occludens; ECJ, endothelial cell-cell junction; P-face, protoplasmic-face; VEGF, vascular endothelial growth factor; JAIL, junction-associated intermediate lamellipodia; YAP, yes-associated protein 1; TAZ, transcription regulator 1; p-MLC, phosphorylated myosin light chain; PECAM-1, platelet endothelial cell adhesion molecule-1; HBMEC, human brain

microvascular endothelial cell; FAJ, focal adherens junction; HUVEC, human umbilical vein endothelial cell; TNF, tumor necrosis factor-α; JAnaP, Junction Analyzer Program; TEM, transendothelial migration; TNBC, triple negative breast cancer; iBMEC, induced brain microvascular endothelial-like cells derived from human induced pluripotent stem cells; IL, interleukin; N. meningitidis, Neisseria meningitidis; mBCFSB, meningeal blood-cerebrospinal fluid barrier; TEER, transendothelial electrical resistance; BAEC, bovine aortic endothelial cell; hiPSC, human induced pluripotent stem cell; PAEC, porcine aortic endothelial cells; MLCK, myosin light chain kinase; ROCK, Rho-associated protein kinase; HLMEC, human lung microvascular endothelial cell; FAK, focal adhesion kinase; HAEC, human aortic endothelial cell; HRMEC human retinal microvascular endothelial cell.

Abstract

Cells depend on precisely regulating barrier function within the vasculature to maintain physiological stability and facilitate essential substance transport. Endothelial cells achieve this through specialized adherens and tight junction protein complexes, which govern paracellular permeability across vascular beds. Adherens junctions, anchored by VE-cadherin and associated catenins to the actin cytoskeleton, mediate homophilic adhesion crucial for barrier integrity. In contrast, tight junctions composed of occludin, claudin, and junctional adhesion molecule A interact with Zonula Occludens proteins, reinforcing intercellular connections essential for barrier selectivity.

Endothelial cell-cell junctions exhibit dynamic conformations during development, maturation, and remodeling, regulated by local biochemical and mechanical cues. These structural adaptations play pivotal roles in disease contexts such as chronic inflammation, where junctional remodeling contributes to increased vascular permeability observed in conditions from cancer to cardiovascular diseases. Conversely, the brain microvasculature's specialized junctional arrangements pose challenges for therapeutic drug delivery due to their unique molecular compositions and tight organization.

This commentary explores the molecular mechanisms underlying endothelial cell-cell junction conformations and their implications for vascular permeability. By highlighting recent advances in quantifying junctional changes and understanding mechanotransduction pathways, we elucidate how physical forces from cellular contacts and hemodynamic flow influence junctional dynamics.

Introduction

The precise delivery of essential circulating components, such as nutrients, endocrine signals, and therapeutic agents, to organ tissues is vital for maintaining physiological balance and stability[1]. Endothelial cells play a central role in this process by lining the vasculature and establishing a barrier that regulates the extravasation of substances into underlying tissues[1,2]. This barrier is primarily maintained by two protein complexes: adherens and tight junctions, each characterized by specific constituent protein-protein interactions[3–5]. Vascular endothelial (VE)-cadherin, a transmembrane protein, facilitates homophilic adhesion between neighboring endothelial cells, initiating adherens junction formation[3]. The cytoplasmic tail of VE-cadherin interacts with proteins such as α-, β-, and p120-catenin, as well as plakoglobin, anchoring adherens junctions to the actin cytoskeleton[3,6].

Similarly, tight junctions exhibit complexity, with transmembrane proteins such as occludin, claudin, and junctional adhesion molecule A facilitating intercellular adhesion[3]. These proteins interact with intracellular counterparts like zonula occludens (ZO)-1/2/3

to reinforce connections to the actin cytoskeleton. Baseline differences in the organization of endothelial cell-cell junction (ECJ) components and protein expression can vary based on the vascular bed. This is evident in freeze-fracture preparations, where tight connections between endothelial cells in blood vessels outside the brain are less associated with the Protoplasmic (P)-face compared to blood vessels of the brain, where these connections are most prominently associated with the P-face[7].

Additionally, occludin exhibits high gene and protein expression levels with a continuous distribution in brain endothelial cells, whereas in endothelial cells of non-neural tissues, its expression is much lower and shows a discontinuous pattern[8]. ECJs can also have different conformations depending on the stage of adhesion: initial formation[9–12], stable maturation [9,13–18], and stimulated remodeling[9,10,19–21]. These conformations are interchangeable, each uniquely characterized by specific local actin organization and associated intracellular proteins. As a result, adherens and tight junctions are thought to undergo conformational changes in response to various biochemical and mechanical signals.

Persistent remodeling of ECJs under chronic inflammatory conditions is thought to contribute to increased endothelial permeability seen in a spectrum of pathologies, including cancer, cardiovascular disease, ischemic stroke, asthma, and arthritis[1]. On the other hand, baseline differences in the expression of adherens and tight junction proteins, along with their stable, well-organized arrangements within the brain microvasculature, are believed to present challenges for targeted drug delivery to brain tissues[7,8,22–24]. Understanding how adherens and tight junction conformations influence endothelial permeability and the mechanisms governing their remodeling could inform strategies for disease management and drug development.

The molecular composition and signaling of adherens and tight junctions[3,4,25–27], along with their heterogeneity throughout the vasculature tree[28–31] and their contribution to paracellular permeability[1,2,32–38], have been extensively documented in previous reviews. In this commentary, we will discuss the various ECJ conformations and how changes in their structure affect barrier permeability. We will describe tools to quantify these changes and their role in immune cell extravasation. Additionally, we will outline the impact of contact and flow-derived forces on cell-cell junction conformations, focusing on mechanotransduction and its influence on permeability function.

Endothelial Cell-cell Junction Conformations

ECJ proteins and baseline permeability exhibit significant diversity across different vascular beds from various anatomical regions[39–41]. This heterogeneity has prompted a comprehensive examination of adherens and tight junctions as regulators of paracellular permeability[3–5]. Researchers have extensively studied the morphology and molecular composition of ECJs using freeze-fracture electron microscopy[42]. These studies reveal

that the number and complexity of junctional strands vary by cell type, which affects the barrier properties of different tissues. Specifically, an increase in the number of junctional strands correlates logarithmically with specific junctional resistance[43]. These finding challenges earlier descriptions of ECJs merely as fusions or seals of the outer membrane leaflets of adjacent cells[43–45]. Instead, it suggests that ECJs contribute to barrier function in a more complex manner, involving various regulatory proteins and influenced by factors such as spatial arrangement and dynamic interactions[3–5,43–45].

As a result, disruption of transmembrane or cytoplasmic proteins in adherens and tight junctions affects permeability. Tissue-specific expression of claudin isoforms and the effects of disease-causing tight junction protein mutations have significantly contributed to our understanding of their role in barrier formation[4,46–48]. For example, claudin-5-deficient mice exhibit size-selective loosening of the blood-brain barrier, impairing its ability to restrict molecules smaller than 800 Daltons[48]. Intravenous injection of the BV13 antibody, which targets mouse VE-cadherin and redistributes it away from adherens junctions, caused a dose- and time-dependent increase in vascular permeability in 10–12-week-old male mice[49]. Knocking out β-catenin in mouse models decreased endothelial cells' ability to maintain vascular integrity, leading to leakage and hemorrhage[50].

In this section, we will discuss the different conformations of cell-cell junctions (Table 1). Given that adherens junctions are ubiquitously expressed along the vascular tree, we will focus primarily on cadherin-containing junctions observed through microscopic analyses[3,51]. We will indicate what is known about their local actin organization and associated intracellular proteins at different stages of adhesion.

The initial formation of ECJs have a distinct conformation that differs from their mature stable structure. This has been illustrated in studies involving the human materno-fetal endothelial barrier where ECJs can display unique conformations linked to vascular changes during pregnancy[52–54]. Developing vessels in the first trimester display an 'activated junctional phenotype,' primed for cell growth and proliferation[52]. Stimulated by the predominant angiogenic growth factor, vascular endothelial growth factor (VEGF), these vessels exhibit ECJs lacking plakoglobin, occludin, and claudin-1 at adherens and tight junction sites compared to the 'stable junctional phenotype' of third-trimester vessels[52,54]. *In vitro* studies using human placental endothelial cells in endothelial conditioned growth supplement confirmed this 'activated junctional phenotype,' marked by a more 'punctate' morphology of occludin, reduced plakoglobin and β-catenin at adherens junctions, and corresponding changes in F-actin organization[53,54].

Initial forming cell-cell junctions are thin and discontinuous, assuming a punctate morphology[9,11,12] (Table 1). In subconfluent and migratory endothelial models, junction-associated intermittent lamellipodia (JAIL) protrusions mediate the interaction between endothelial cells, which retract and transform into filopodia-like bridges rich in VE-

cadherin[11]. The conformations of VE-cadherin along these bridges are heterogeneous, with multiple punctate accumulations in an interrupted pattern[11,12]. Non-muscle myosin II incorporates into these bridges, maturing them into stress fibers [11]. Many proteins associate with forming cell-cell junctions, mediating their function and connection to actin, including VASP proteins, fascin, ARP 2/3 complex, α-catenin, and β-catenin[9–12].

ECJs stabilize and mature after their initial formation, adopting a thick, continuous, and linear morphology in highly confluent monolayers, endothelial cells under laminar flow, or with increased cyclic AMP[9,13–15] (Table 1). This stabilization is accompanied by actin cytoskeleton remodeling. Actin filaments become shorter and more irregular, colocalizing with linearly distributed VE-cadherin and forming peri-junction actin bundles, where VE-cadherin aligns parallel to circumferentially organized actin networks[13,18,21]. Stable ECJs retain α-catenin and β-catenin, with plakoglobin accumulating at maturing adherens junctions as endothelial cells near confluency[9,18].

Reticular adherens junctions are a unique and stable ECJ conformation, identified as a 3-dimensional network formed by overlapping quiescent endothelial cells[16] (Table 1). Transcription factors yes-associated protein 1 and transcription regulator 1 (YAP/TAZ) are required for these VE-cadherin reticular structures[55]. Knockdown studies of p73 in endothelial cells, recently identified as a regulator of YAP, confirmed its role in the formation and maintenance of reticular junctions [56]. Early tyrosine kinase SRC activation also stimulates the formation of reticular junctions, enhancing endothelial barrier function via phosphorylation of VE-cadherin at Y731[57].

While VE-cadherin can be distributed into reticular structures, tight junction proteins like ZO-1 do not appear in this conformation[16]. Unlike other ECJ conformations, reticular junctions have little to no attachment to actin[16]. Additionally, common endothelial cell tension markers like phosphorylated myosin light chain (p-MLC) and vinculin are absent in reticular structures, suggesting this ECJ conformation forms in regions under minimal mechanical tension[16]. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is found in specific regions within reticular junctions, contributing to their stability and regulating their permeability through β-catenin[16,58]. Several adherens junction proteins, including α-catenin, β-catenin, and p120-catenin, are also distributed in reticular structures[16].

The versatility of endothelial cells stems from their unique ability to detect and respond to diverse inputs, including mechanical and chemical signals, and to produce various outputs accordingly. Thus, stable ECJ conformations can remodel in response to environmental changes. For instance, the transmigration of small cell lung cancer cells (NCI-H209) across human brain microvascular ECs (HBMECs) was accompanied by changes in tight junction morphology[59]. The increase in NCI-H209 cell transendothelial migration coincided with alterations in cytoskeletal actin and ECJ conformations (e.g., ZO-1, occludin, and claudin-5). This led to the transformation of stable, continuous, linear junctions into "discontinuous," "segmented," and "dotted" structures[59]. Inhibition of

Rho kinase with Y27632 prevented changes in local actin organization and ECJ conformations, thereby impeding NCI-H209 transmigration.

During processes such as endothelial wound healing, cell migration, and inflammation, continuous adherens junctions can transition into a remodeling, discontinuous structure known as 'focal adherens junctions' (FAJs)[9,10,15,19–21] (Table 1). To study this transition, researchers used time-lapse imaging of human umbilical vein endothelial cells (HUVECs) expressing α-catenin tagged with a photoswitchable fluorescent protein called Dendra2[21]. Dendra2 changes its fluorescence color when exposed to specific wavelengths of light—a process referred to as “photoswitching”. In these experiments, a segment of a stable junction was photoswitched from green to red fluorescence using a 405-nm confocal laser. Thrombin was then applied to induce FAJ formation, allowing observation of whether the photoswitched α-catenin molecules were retained or replaced during the transition from stable to remodeling FAJs[21]. This approach revealed that a significant fraction of the photoswitched α-catenin molecules remained associated with the junctions, indicating that FAJs are formed by remodeling existing adherens junctions. This remodeling involves molecular and physical changes, including the recruitment of vinculin, actin-regulatory proteins such as VASP, zyxin, and TES, and the binding of radial actin to cadherin complexes, all of which persists throughout the transition[20,21].

Tumor necrosis factor (TNF), a well-established activator of Rho, exerts significant effects on endothelial cells during both early and late phases of stimulation[10]. Early exposure to TNF induces changes in the actin cytoskeleton of HUVECs, promoting the formation of stress fibers[10,60]. In contrast, prolonged TNF exposure triggers Rho-independent remodeling of ECJs, leading to increased permeability. In TNF-treated endothelial cells, VE-cadherin, α-catenin, and β-catenin complexes exhibit a discontinuous pattern, characterized by breaks within regions of stable linear junctions[61]. These breaks manifest as short linear structures that branch off from continuous ECJs, often attaching to the ends of stress fibers rather than to cortical F-actin[61].

In summary, ECJs exhibit diverse conformations across various vascular contexts, influenced by physiological and pathological stimuli[59,62–67]. From stable to remodeling forms like FAJs, these junctional structures undergo dynamic molecular and physical changes involving actomyosin cytoskeleton remodeling and recruitment of specific intracellular proteins. Understanding these variations provides insights into how endothelial barriers respond to mechanical and biochemical cues, essential for maintaining vascular integrity and adapting to physiological demands.

Quantitative Evaluation of Cell-cell Junction Conformations

Each cell type responds uniquely even to the same physiological stimulus, exhibiting distinct characteristics[68–70]. Various stimuli can disrupt junction architecture to varying extents, ranging from minor changes in protein composition to complete loss of adhesive homophilic transmembrane contacts and associated cytoplasmic proteins[71]. Despite observed alterations in the structural presentation of ECJs, the precise regulatory mechanisms governing these changes remain elusive. Understanding the conditions under which these regulatory processes occur, particularly across different vascular beds, requires further investigation. A significant challenge in studying ECJs comprehensively is the current limitations of tools and methodologies used for quantification, which often struggle to identify and analyze the nuanced conformations of these junctions effectively.

Software tools like ImageJ are commonly used to measure various characteristics of ECJs, such as junction gap width and linearity[21,62,72]. To assess junction gap width, indicative of potential barrier dysfunction, a manual line is drawn perpendicular to the widest gap visible in a fluorescent image of a junction (Fig. 1A). The software then generates a pixel intensity profile for this designated area. Linearity is evaluated by manually measuring the lengths of adherens junctions that display both linear and non-linear staining patterns using the line tool. Typically, the percentage of linear junctions is determined by comparing the length of linear junctions to the total junction coverage.

Existing tools predominantly rely on analyzing immunostaining intensity in images, but they often overlook critical features such as junction shape, fragmentation, and continuity—essential aspects that can significantly affect function and are observable through microscopic examination. Moreover, these tools require manual input, leading to time-consuming analyses and potential bias from users. These inherent challenges in quantifying ECJs have historically hindered systematic studies of mammalian cells, exacerbated by issues like cytoplasmic noise and irregular cell edges[73]. As a result, qualitative assessments based on the presence of junctional proteins at cell-cell interfaces have dominated the literature. Recently, efforts to address these limitations have led to the development of two semi-automatic programs: the Junction Mapper Program by Brezovjakova et al. and the Junction Analyzer Program (JAnaP) developed by our laboratory[65,71].

Junction Mapper and JAnaP both start their analysis by creating a skeleton outline of cell edges based on pixels at junction contact points identified through fluorescent labeling. Junction Mapper autonomously determines these edges, allowing manual adjustments by users if needed. In contrast, JAnaP requires users to mark waypoints along the cell edge, after which the program automatically connects these markers at cell-cell interfaces. These skeletal outlines serve as the basis for calculating various parameters to quantify ECJs.

Junction Mapper provides primary parameters such as junction area, contour, and straight-line length, alongside secondary parameters that standardize the primary metrics based on junction size or contour interface (Fig. 1B). On the other hand, JAnaP automates the calculation of diverse cell morphology parameters including area, perimeter, circularity, and solidity. Additionally, JAnaP quantitatively categorizes different junction types—such as continuous, perpendicular, and punctate—showing their distribution along the entire cell edge (Fig. 1C).

Each program offers distinct advantages. Junction Mapper's primary parameters allow for assessing junction shrinkage, structural changes, and continuity based on marker intensity within cell-cell contact zones, a feature less emphasized in JAnaP. However, Junction Mapper faces challenges in accurately outlining the contour of zig-zag junctions at interfaces, which are characteristic of activated or remodeling junction configurations. In contrast, JAnaP excels in calculating detailed tip-to-tip distances for each junction feature along the cell edge, making it particularly valuable for evaluating disruptions that result in zig-zag patterns.

In summary, both Junction Mapper and JAnaP represent significant advancements in objectively quantifying junctional changes, addressing longstanding challenges in cell junction research. Each program offers distinct analytical strengths: Junction Mapper provides a comprehensive array of parameters to characterize junctional alterations induced by diverse stimuli, generating specific profiles. In contrast, JAnaP quantifies junctions based on conformations commonly described qualitatively in the literature. This makes JAnaP particularly valuable for quantifying junction parameters that complement qualitative observations, thereby enhancing established studies.

These semi-automated analytical techniques are relatively recent developments, introduced within the past five years, and have seen limited application in existing literature. Consequently, the following discussion primarily involves qualitative observations of ECJ conformations. Among the relevant studies that have employed these semi-automatic tools, we focus on their findings regarding the effects of inflammatory and mechanical challenges on junctional integrity.

Cell-Cell Junction Conformations and Transendothelial Migration

Transendothelial migration (TEM) of various cell types is a critical aspect of both normal and pathological processes. The barrier properties of the endothelium are central to regulating this migration. In particularly, the impact of ECJ forms can alter the rate of TEM in cells crossing the endothelial barrier via the paracellular route. For example, the transmigration of triple negative breast cancer (TNBC) cells, MDA-MB-231, across the brain microvascular endothelial-like cells derived from human induced pluripotent stem cells (iBMEC-like cells), increased with interleukin (IL)-1β pretreatment[74]. To analyze junction integrity using JAnaP, confocal images of iBMEC-like cells stained for both ZO-

1 and claudin-5 were captured after 6 hours of IL-1β treatment. IL-1β reduced the percentage of continuous coverage (from ~96% to ~82%, $p<0.001$) and increased the percentage of punctate (from ~1% to ~7%, $p<0.001$) and perpendicular (from ~1% to ~2%, $p<0.01$) regions within the cell–cell junctions for claudin-5, but not ZO-1 despite a downregulation of mRNA expression[74]. IL-1β neutralizing antibodies reduced the transmigration of TNBC cells. These findings suggest that the increased TEM induced by IL-1β is mediated by the changes in claudin-5 from a more stable cell-cell junction conformation to a more activated or remodeled conformation.

IL-1β has also been shown to activate endothelial cells by prompting the expression of various receptors on the endothelial surface, stimulating the release of cytokines and inducing a procoagulant endothelial phenotype that influences permeability[75]. An investigation by Burns et al. revealed that IL-1β pretreatment of HUVEC monolayers increased the percent TEM of neutrophils (65.8 ± 5.4 vs 0.1 ± 0.1, $p<0.05$)[76]. Of the fraction of neutrophils traversing the endothelial monolayer, over 75% cross at tricellular junctions—points of intersection between three endothelial cells—compared to less than 25% at bicellular junctions (between adjacent endothelial cells)[76]. Similarly, Dias et al. observed a comparable trend with T-cells, which predominantly passed paracellularly (around 79.4%) through primary mouse brain microvascular endothelial cells, with more than 60% passing through tricellular junctions[77]. Notably, junctions at tricellular regions have been observed to exhibit a discontinuous conformation both *in vitro* and *ex vivo*[78–80]. This pattern suggests that the inclination for neutrophil and T-cell TEM at tricellular junctions (with a discontinuous form), as opposed to bicellular junctions (with an intact structure), might arise from a path of least resistance[76].

Quantifying the percentage of ECJ coverage has shown that reductions in total junction coverage can impact the rate of paracellular transport across the endothelial barrier. An illustration of this can be seen with gram-negative bacterium, Neisseria meningitidis. N. meningitidis is the leading cause of bacterial meningitis worldwide and requires the traversal of the meningeal blood-cerebrospinal fluid barrier (mBCFSB), composed in part by brain endothelial cells[81,82]. An in-vitro model of bacteria traversing the mBCFSB suggested a transcellular route for N. meningitidis as bacteria transmigrated the barrier within 24-hours post-infection, as barrier integrity, measured by transendothelial electrical resistance (TEER), was still near control levels[83].

Interestingly, the rates of bacterial transmigration across brain endothelial cells increased significantly between 24-hour and 30-hours post infection, accelerating well beyond the rate of transmigration during the first 24-hours[83]. Analysis with JAnaP revealed that occludin coverage at cell junctions significantly decreased from approximately 75% to 45% between 24- and 30-hours post-infection, indicating that N. meningitidis likely traverses the brain endothelium through a paracellular pathway when it is accessible. These observations collectively highlight the critical role of junctional integrity and conformation in facilitating TEM, emphasizing how specific molecular and

structural changes within ECJs can significantly impact the rate and pathway of cellular and microbial transmigration.

Forces on Cell-cell Junction, Expression, Conformations, and Permeability

Mechanotransduction is the intricate process by which endothelial cells sense and convert biomechanical forces into intracellular signals, influencing cellular positioning and behavior[84]. ECJs have been found to play a crucial role in this process by sensing and transmitting mechanical signals, which can be disrupted in conditions affecting vascular mechanics, impacting both normal physiology and disease outcomes[85,86]. For instance, shear stress promotes the maturation of the vascular barrier by enhancing junction linearity and stability[87]. VE-cadherin, located at cell-cell junctions, experiences significant myosin-dependent tension under normal conditions, which rapidly decreases (<30 seconds) in response to shear stress, reducing overall cell-cell tension[88].

Our laboratory previously reviewed various mechanical forces affecting endothelial cell behavior[85]. Here, the subsequent discussion focuses on both qualitative and quantitative analyses of how ECJs structurally respond to mechanical cues and their implications for vascular permeability (summarized in Table 2).

CYCLIC STRAIN

The impact of cyclic strain on ECJs varies depending on the type of endothelial cell. For instance, physiological levels of cyclic strain (5% strain for 12 or 24 hours) decreased mRNA expression of occludin in HUVECs but increased its mRNA expression in bovine aortic endothelial cells (BAECs)[68,70]. Additionally, in unstrained BAECs, occludin localization at junction sites was low, but exposure to physiological levels of cyclic strain increased its junction coverage[70]. ZO-1 showed a discontinuous and jagged pattern at the cell edge in unstrained endothelial cells, which gradually matured into a more stable conformation with continuous, well-defined junctions upon application of cyclic strain[70].

Tight junction assembly is highly regulated and involves various signaling pathways, including the activity of protein kinase C [89,90]. Inhibition of protein kinase C with rottlerin ablated the endothelial cell's response to cyclic strain, causing ZO-1 proteins to revert to a more activated or remodeled conformation (Fig. 2) [70]. The study also showed that unstrained BAECs were more permeable to 40 kDa FITC-dextran than strained cells, suggesting that the effects of cyclic strain, which stabilize ZO-1 conformation, improve barrier function (Table 2).

In a 3D-vessel-on-chip model using a fluidic circuit, human induced pluripotent stem cell (hiPSC)-derived endothelial cells fluorescently labeled for VE-cadherin displayed a continuous morphology under pressure-induced circumferential strain[91]. At 0 mbar, the VE-cadherin maintained a linear orientation, which persisted up to 100 mbar of internal

pressure[91]. However, at 150 mbar (~2% strain), VE-cadherin exhibited an activated or remodeling junction orientation, observed as a zigzag pattern (Table 2)[91]. These zigzag discontinuous patterns at 150 mbar could potentially influence the permeability of the endothelium, though this effect was not directly measured in this new model.

Although limited studies have investigated the response of junction structures and conformations to cyclic strain, these few studies provide valuable insights into the dynamic regulation of ECJs under this mechanical stress. This aspect remains understudied, highlighting the need for further research to fully understand these mechanisms and how they vary across endothelial cell types.

MATRIX STIFFNESS

In endothelial cells, substrate stiffness plays a crucial role in various physiological and pathological conditions, including aging, atherosclerosis, solid tumors, and neurodegenerative disorders. Extensive research across *in-vitro*, *ex-ovo*, and *in-vivo* studies has demonstrated that changes in substrate stiffness significantly influence vascular permeability[6,92]. These alterations in permeability often coincide with changes in junction conformations, reflecting endothelial cell adaptive responses to their mechanical microenvironment. For instance, elevated levels of matrix stiffness (15 kPa and 194 kPa) have been shown to promote continuous ZO-1 coverage at junctions in iBMEC-like cells[93]. This increase in ZO-1 coverage correlated with a notable nine-fold reduction in FITC-avidin permeability in a localized permeability assay conducted on stiffer substrates. In contrast, on a more compliant substrate (1 kPa), where ZO-1 and Claudin-5 were more discontinuous, the permeability was higher (Table 2)[93]. Interestingly, this reduction was most pronounced at tricellular junctions within the monolayer.

BAECs and HUVECs cultured on compliant matrices (e.g., 0.2 kPa) typically develop continuous adherens junctions and tight junctions along their cell peripheries[94]. In contrast, stiffer matrices (e.g., 10 kPa) induce a punctate morphology of these junctions. This change in junction protein structure due to matrix stiffness correlates directly with increased permeability, as demonstrated by enhanced passage of 40-kDa FITC dextran molecules (Fig. 2)[94]. On softer substrates (6 kPa), primary porcine aortic endothelial cells (PAECs) exhibit thick reticular adherens junctions[92]. Conversely, when PAECs are cultured on stiffer substrates (29 kPa), these reticular structures become thinner and are lost. Moreover, PAEC monolayers exposed to stiffened substrates and inflammatory cytokines demonstrate enhanced vinculin accumulation at adherens junctions, increased tension, and elevated permeability[92].

The cell contractility pathway is a well-established mechanism linking matrix stiffness, ECJs, and endothelial permeability. Studies employing techniques like traction force microscopy have shown that endothelial cell contractility increases in response to stiffer

matrices [72,95]. This heightened contractile response is often associated with activation of myosin light chain kinase (MLCK) and subsequent p-MLC. These actions generate centripetal forces, widening and disrupting junctions, thereby promoting the TEM of immune cells, particularly neutrophils, via a paracellular route[92,96,97].

RhoA, another potent activator of the cell contractility pathway, targets the actin cytoskeleton through effectors such as Rho-associated protein kinase (ROCK)[98]. ROCK's mechanism involves inhibiting myosin light chain phosphatase, leading to increased p-MLC downstream[98]. While myosin light chain kinase (MLCK) plays a more significant role in endothelial cell hyperpermeability than ROCK, mild inhibition of ROCK has been shown to restore impaired endothelial cell monolayers cultured on stiffer matrices[72,99]. Inhibiting ROCK reduces permeability and leukocyte transmigration by mitigating the stiffness-dependent increase in adherens junction width (destabilization), highlighting the critical role of junction conformation in regulating permeability[72].

The influence of matrix stiffness on junction conformation extends from large vessels to smaller ones. Human lung microvascular endothelial cells (HLMECs) demonstrate distinct responses when cultured on matrices with varying stiffness levels. Cells on low (150 Pa) and high (35 kPa) stiffness matrices exhibit disrupted and discontinuous VE-cadherin junctions, correlating with increased permeability (Table 2)[100]. Conversely, cells cultured on an intermediate stiffness (4 kPa) show fewer discontinuities and higher TEER measurements[100]. This study underscores the delicate balance between cellular contractility and relaxation responses, crucial for optimal junction formation and stability, thereby ensuring the integrity of the endothelial barrier (Fig. 2).

HBMECs typically reside in a microenvironment characterized by a soft, hyaluronic acid-rich extracellular matrix with low stiffness (0.1-1 kPa)[101]. In diseased states, alterations in the crosslinking of extracellular matrix proteins often increase matrix stiffness, promoting disease progression[102]. HBMECs cultured on hyaluronic/gelatin films with varying Extralink concentrations (0.2%, 0.8%, 1.2%, and 2%) exhibited stiffness measurements of 0.85 kPa, 1.1 kPa, 1.5 kPa, and 3.8 kPa, respectively[66]. HBMECs cultured on films with 1.1 kPa stiffness displayed the highest percentage of stable continuous, initially forming punctate, and activated perpendicular ZO-1 conformations simultaneously[66]. However, these changes in conformation were modest and did not correlate with alterations in cancer cell transmigration speed or incorporation[66]. This lack of correlation is likely due to the stiffness range still being within healthy physiological limits.

In addition to the cell contractility pathway, other mechanisms contribute to matrix-induced changes in ECJ conformations and permeability. A study using HUVECs cultured on collagen-1-coated polyacrylamide hydrogels demonstrated that matrix stiffness directly influences focal adhesion kinase (FAK)-mediated regulation of permeability[6]. Increased matrix stiffness heightened FAK activity, leading to modulation

of ECJ width without altering total VE-cadherin protein levels. Instead, FAK activation induced junctional disruption by phosphorylating VE-cadherin, facilitated by Src translocation to cell junctions, thereby reducing β-catenin presentation at the intercellular cleft and causing discontinuities[6]. These changes correlated with increased endothelial permeability, which was mitigated by FAK inhibition. This study suggests that matrix stiffness contributes to heightened permeability through increased FAK activity and junctional disruption.

In summary, the endothelial monolayer's response to matrix stiffness activates various signaling cascades that influence ECJ conformation and vascular barrier integrity. These insights illuminate the mechanobiological principles governing vascular function and offer potential for developing therapeutic strategies targeting these pathways[103].

SHEAR STRESS

The maturation of initially forming ECJs can be modulated by shear stress within the physiological range, which may vary depending on the endothelial cell type[85]. Long-term culturing of endothelial cells under physiological laminar flow promotes junctional conformations typical of mature endothelium. For instance, VE-cadherin in human aortic endothelial cells (HAECs) and HUVECs exhibits a mature linear pattern at 6 dyn/cm², while ZO-1 in HBMECs shows a similar pattern at 10-20 dyn/cm², with actin localized to the cell perimeter[104,105]. Conversely, in the absence of flow (static controls) or under abnormally high shear stress (e.g., 40 dyn/cm² in HBMECs), ZO-1 translocates to the cytoplasm, resulting in a discontinuous junction pattern (Table 2)[105]. This indicates that homeostatic shear flow is optimal for junction stability and barrier function.

A study using primary human retinal microvascular endothelial cells (HRMECs) further supports this, showing ZO-1 with the most stable linear conformation at 5 dyn/cm² shear stress[106]. As shear stress levels increased (>10 dyn/cm²) or decreased (<1.5 dyn/cm²), ZO-1 distribution at junctions decreased, resulting in a discontinuous pattern (Table 2, Fig. 2). Variations in shear stress are widely recognized as significant contributors to changes in endothelial permeability, influencing the development of atherosclerotic plaques in cardiovascular disease[107,108]. The emergence of plaques in areas with disturbed flow patterns, such as branched and curved regions, raises questions about the impact of these biomechanical cues on endothelial behavior, junction stability, and subsequent permeability.

In adherens junctions, VE-cadherin staining at endothelial cell borders in vivo varied significantly depending on the type of flow conditions. In the descending thoracic aorta, where laminar pulsatile flow exhibited a predominant net forward component, VE-cadherin staining was notably stronger compared to the curved aortic arch, where flow near the wall was fluctuating and reciprocating with minimal net forward flow[109]. Using flow chambers to simulate these conditions in vitro, BAEC monolayers exposed to

pulsatile flow (12 ± 4 dyn/cm² at 1 Hz) or reciprocating flow (0.5 ± 4 dyn/cm² at 1 Hz) for 6 hours showed discontinuous VE-cadherin staining along cell borders, contrasting with the continuous VE-cadherin distribution observed in static controls[109]. Extending pulsatile flow exposure to 24, 48, or 72 hours restored the more stable continuous VE-cadherin conformation, whereas staining remained intermittent with prolonged reciprocating flow exposure under similar conditions.

Proatherogenic multidirectional or disturbed flow conditions have been associated with promoting endothelial hyperpermeability. In an experiment, PAECs were cultured in a multi-well plate placed on an orbital platform shaker[107]. This setup exposed PAECs at the center to multidirectional flow and those at the edge to unidirectional flow. Cells exposed to multidirectional flow had a higher percentage of leaky VE-cadherin tricellular junctions compared to cells under unidirectional flow (~27% vs ~15%), which are known to exhibit a discontinuous conformation (Table 2)[78–80,107]. Additionally, cells under multidirectional flow showed approximately a two-fold increase in the passage of FITC-avidin through their tricellular junctions compared to their unidirectional flow-stimulated counterparts (Fig. 2)[107].

This was also observed in a cerebral bifurcation 3D in-vitro model, where brain hCMEC/D3 endothelial cells exposed to disturbed flow conditions for 18 hours underwent changes in local actin organization, forming stress fibers and displaying more discontinuous ZO-1 conformations compared to cells under fully developed unidirectional flow[110]. The disturbed flow-conditioned cells had a permeability coefficient of approximately $3x10^{-6}$ cm/s (for 4 kDa FITC dextran), whereas the fully developed flow-conditioned cells had a permeability coefficient of around $0.5x10^{-6}$ cm/s (Table 2)[110].

In summary, the data discussed demonstrate that different shear stress conditions significantly influence endothelial cell junction conformation and permeability. Physiological shear stress promotes stable, continuous junctions, while disturbed or high shear stress leads to discontinuous junction patterns and increased permeability. These findings highlight the importance of biomechanical forces in regulating endothelial barrier function and underscore the need for further research to fully understand the mechanisms driving these changes in a more quantitative manner.

Conclusions and Future Directions

Understanding the conformational dynamics of ECJs and their implications in various physiological and pathological contexts underscores their pivotal role in regulating vascular permeability. The diversity in ECJ protein composition and baseline permeability across different vascular beds highlights the intricate nature of ECJ-permeability relationships. The adoption of semi-automatic programs such as Junction Mapper and JAnaP for more comprehensive ECJ quantification promises to advance vascular mechanobiology significantly. These tools will be instrumental as we continue

to uncover the mechanistic roles of mechanical forces in modulating ECJ conformations and vascular permeability.

A critical challenge moving forward is the development of robust in-vitro experimental models that can effectively explore the impact of mechanical stimuli on ECJ conformations and permeability. It is essential to create in-vitro setups capable of directly measuring permeability immediately following the application of mechanical signals. A recent noteworthy innovation in this realm is the microfluidic electrochemical assay pioneered by Jeremy F. Wong and Craig A. Simmons[111]. This approach integrates mechanical cues, particularly shear stress, and enables direct measurement of permeability, marking a significant advancement in studying ECJ responses to biomechanical stimuli.

This technology differs from traditional methods that use fluorescent tracers by employing an electroactive tracer and integrating electrodes in the lower channel. Similar to a Transwell insert, a porous membrane separates upper and lower channels where endothelial cells are cultured. This configuration allows researchers to observe real-time diffusive or convective transport of the electroactive tracer through the monolayer, a capability lacking in traditional Transwell systems. Concurrently, TEER facilitates real-time monitoring of barrier function, though interpreting results has historically posed challenges. This technological advancement is crucial as it offers insights into how mechanical cues, such as shear stress, immediately influence endothelial barrier function and permeability. It fosters a deeper understanding of the dynamic changes in vascular function.

In contrast, cyclic strain stimulation and permeability analysis are typically conducted sequentially in conventional approaches. Endothelial cells undergo cyclic strain, followed by disruption of their protein complexes through trypsinization. Subsequently, they are re-seeded onto membranes for permeability studies using conventional Transwell systems[70]. This approach introduces delays in permeability measurements and introduces confounding variables as endothelial cells need to re-adhere to form a new monolayer on the Transwell membrane. Moreover, TEER and Transwell assays provide bulk quantitative permeability measurements, thereby not allowing for localized permeability differences within the monolayer (e.g., bicellular, tricellular junctions) to be discerned. Dubrovskyi et al. developed an alternative method that utilizes the culture surface itself as the permeable detection surface[112]. They achieved this by biotinylating the substrate (e.g., fibronectin, collagen, or gelatin) with EZ-link NHS-LC-LC-Biotin and using a fluorescently labeled avidin ligand tracer.

This method involves identifying permeable regions locally as a ligand binds to receptors beneath the cell monolayer. By coating flexible-bottomed culture plates with biotinylated gelatin, researchers measured permeability in human pulmonary artery endothelial cells exposed to cyclic strain. This approach has also been adapted to

assess permeability in response to various mechanical stimuli, such as shear stress[113,114] and matrix stiffness[93]. Consequently, studies enabling immediate analysis of permeability following mechanical stimulation could offer valuable insights into the complex interplay among mechanical forces, endothelial cell junctions, and permeability. Such insights would illuminate the pivotal role of these interactions in vascular physiology and pathophysiology.

Another major challenge is the concurrent implementation of various mechanical cues, as they do *in vivo*, to elicit a complex EC response. For instance, Zhao et al. investigated the combined effects of cyclic strain and shear stress on bovine aortic ECs, revealing that the two stimuli can lead to increased cell alignment (with respect to the flow/shear stress direction) and aspect ratio (the ratio between the cell's long and short axis), demonstrating a synergistic relationship[115]. However, human coronary artery ECs (HAECs) responded differently, showing no significant effects on cell aspect ratio[116]. HAECs showed an increased but not synergistic effect on ICAM-I expression[116]. In contrast, HUVECs subjected to shear stress exhibited downregulated ICAM-I expression when cyclic strain signals were added, indicating an antagonistic relationship[117]. Understanding the impact of multiple mechanical cues is crucial because they interact in complex ways to influence EC behavior, and these interactions can vary depending on the specific EC type and context. Thus, investigating how ECJ conformations and vascular permeability are affected by the interplay of multiple mechanical cues represents a largely unexplored area with the potential to provide fundamental insights and innovative approaches for modulating the vascular endothelial barrier.

Another significant challenge lies in concurrently applying multiple mechanical cues, mirroring physiological conditions in vivo, to elicit a complex endothelial cell response. For example, Zhao et al. explored the combined effects of cyclic strain and shear stress on BAECs, demonstrating that these stimuli synergistically increase cell alignment with the flow direction and aspect ratio, highlighting a synergistic relationship[115]. However, HAECs responded differently, showing no significant changes in cell aspect ratio[116]. Instead, the combined effects of cyclic strain and shear stress increased ICAM-I expression[116]. When subjected to shear stress alone, HUVECs exhibited reduced ICAM-I expression, which was reversed when cyclic strain was added, suggesting an antagonistic interaction[117]. Understanding these complex interactions is crucial because they profoundly influence endothelial cell behavior, with outcomes varying depending on the cell type and specific conditions. Therefore, investigating how ECJ conformations and vascular permeability respond to the interplay of multiple mechanical cues remains a largely unexplored area that holds promise for uncovering fundamental insights and innovative strategies to modulate vascular endothelial barriers.

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Table Legends

Table 1. Overview of different VE-cadherin conformations in endothelial cells, including descriptive changes in local actin organization and associated intracellular proteins. Unique *in-vitro* models that allowed for the observation of these conformations are also described. Actin fibers are depicted in red, while VE-cadherin is marked in green.

Table 2. Summary outlining the responses of endothelial cell-cell junctions to various mechanical stimuli (first column) and describing alterations in vascular barrier permeability (fourth column).

Table 2

Mechanical Stress	Cell Type	Cell-cell Junction Conformation	Permeability	Reference
Cyclic Strain (0%, 24 hrs.)	BAECs	Discontinuous ZO-1 & Occludin	Increased [2.5 fold] (40 kDA FITC-dextran)	Collins et al., 2006
Cyclic Strain (5 %, 24 hrs.)	BAECs	Linear, Continuous ZO-1 & Occludin	Constant (40 kDA FITC-dextran)	Collins et al., 2006
Cyclic Strain (<100 mbar, 24 hrs.)	hiPSC-derived endothelial cells	Linear, Continuous VE-cadherin	n/a	Graaf et al., 2022
Cyclic Strain (>150 mbar, 24 hrs.	hiPSC-derived endothelial cells	Discontinuous VE-cadherin	n/a	Graaf et al., 2022
Matrix Stiffness (>15 kPa)	iBMEC-like cells	↑ Linear, Continuous ZO-1, Claudin-5	Low (<200ng/mL FITC-avidin)	Yan et al., 2023
Matrix Stiffness (1 kPa)	iBMEC-like cells	↑ Discontinuous ZO-1, Claudin-5	High (~900ng/mL FITC-avidin)	Yan et al., 2023
Matrix Stiffness (0.2 kPa)	BAECs, HUVECs	Continuous VE-cadherin	Low (40 kDA FITC-dextran)	Bordeleau et al., 2023
Matrix Stiffness (10 kPa)	BAECs, HUVECs	Discontinuous "Punctate" VE-cadherin	Increased [~3 fold] (40 kDA FITC-dextran)	Bordeleau et al., 2023

Matrix Stiffness (6 kPa)	PAECs	Thick Reticular Adherens Junctions	Mild (TMR-dextran)	Urbano et al., 2017
Matrix Stiffness (29 kPa)	PAECs	Loss and thinning of Reticular Structure	Increased (TMR-dextran)	Urbano et al., 2017
Matrix Stiffness (150 Pa)	HLMECs	↑ Discontinuous VE-cadherin	Increased (TEER < 25 $\Omega \times cm^2$)	Mammoto et al., 2013
Matrix Stiffness (4 kPa)	HLMECs	Continuous VE-cadherin	Low (TEER > 100 $\Omega \times cm^2$)	Mammoto et al., 2013
Matrix Stiffness (35 kPa)	HLMECs	Discontinuous VE-cadherin	Intermediate (TEER ~60 $\Omega \times cm^2$)	Mammoto et al., 2013
Matrix Stiffness (2.5 kPa)	HUVECs	Thin Continuous VE-cadherin	Intermediate (40 kDA FITC-dextran)	Wang et al., 2019
Matrix Stiffness (10 kPa)	HUVECs	↑ VE-cadherin Disruption & Width	Increased [~2-fold] (40 kDA FITC-dextran)	Wang et al., 2019
Shear Stress (6 dyn/cm^2, 48 hrs.)	HAECs, HUVECs	Linear, Continuous VE-cadherin	n/a	Silvani et al., 2021
Shear Stress (10-20 dyn/cm^2, 96 hrs.)	HBMECs	Linear, Continuous ZO-1	n/a	Garcia-Polite et al., 2017
Shear Stress (40 dyn/cm^2, 96 hrs.)	HBMECs	Discontinuous ZO-1	n/a	Garcia-Polite et al., 2017
Shear Stress (5 dyn/cm^2, 24-48 hrs.)	HRMECs	Linear, Continuous ZO-1	n/a	Molins et al., 2019
Shear Stress (<1.5 or >10 dyn/cm^2, 24-48 hrs.)	HRMECs	Discontinuous ZO-1	n/a	Molins et al., 2019
Shear Stress (Unidirectional flow, 7- days)	PAECs	↔ Discontinuous VE-cadherin	Intermediate (FITC-avidin)	Ghim et al., 2022
Shear Stress (Multidirectional flow, 7- days)	PAECs	↑ Discontinuous VE-cadherin	High [~2 fold ↑] (FITC-avidin)	Ghim et al., 2022
Shear Stress (Disturbed flow, 18 hrs.)	hCMEC/D3	Discontinuous ZO-1	Permeability Coefficient $3x10^{-6}$ cm/s (4 kDA FITC dextran)	Bouhrira et al., 2021
Shear Stress (Fully developed flow, 18 hrs.)	hCMEC/D3	Continuous ZO-1	Permeability Coefficient $0.5x10^{-6}$ cm/s (4 kDA FITC dextran)	Bouhrira et al., 2021

Figure Legends

Fig. 1. Computational Tools for Quantitative Assessment of Diverse Cell-Cell

Junctions. A comparison of prominent software tools used for cell-cell junction analysis, highlighting their key features. The red "x" does not necessarily indicate an inability to perform a task but rather evaluates the feasibility and common utilization, or lack thereof, in the literature.

Fig. 2. Impact of Contact-Derived and Flow-Derived Stresses on Cell-Cell Junction Conformations and Permeability. Shear Stress | The flow rate and direction (uni-directional vs. multi-directional) modulate cell-cell junction conformation and tricellular junction leakiness. Cyclic Strain | The effect of physiological strain on cell-cell junctions and permeability is dependent on PKC. Matrix Stiffness | Elevated stiffness levels correlate with changes in cell-cell junction conformations and barrier integrity, varying across different endothelial cell types.

References

1. Claesson-Welsh L, Dejana E, McDonald DM. Permeability of the Endothelial Barrier: Identifying and Reconciling Controversies. *Trends Mol Med*. 2021;27(4):314-331. doi:10.1016/J.MOLMED.2020.11.006

2. Claesson-Welsh L. Vascular permeability - The essentials. *Ups J Med Sci*. 2015;120(3):135-143. doi:10.3109/03009734.2015.1064501

3. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: Molecular organization and role in vascular homeostasis. *Physiol Rev*. 2004;84(3):869-901. doi:10.1152/PHYSREV.00035.2003/ASSET/IMAGES/LARGE/Z9J0030403181006.JPEG

4. Dejana E. Endothelial cell-cell junctions: Happy together. *Nat Rev Mol Cell Biol*. 2004;5(4):261-270. doi:10.1038/nrm1357

5. Dejana E, Orsenigo F, Molendini C, Baluk P, McDonald DM. Organization and signaling of endothelial cell-to-cell junctions in various regions of the blood and lymphatic vascular trees. *Cell Tissue Res*. 2009;335(1):17-25. doi:10.1007/s00441-008-0694-5

6. Wang W, Lollis EM, Bordeleau F, Reinhart-King CA. Matrix stiffness regulates vascular integrity through focal adhesion kinase activity. *FASEB Journal*. 2019;33(1):1199-1208. doi:10.1096/fj.201800841R

7. Wolburg H, Lippoldt A. Tight junctions of the blood–brain barrier: development, composition and regulation. *Vascul Pharmacol*. 2002;38(6):323-337. doi:10.1016/S1537-1891(02)00200-8

8. Hirase T, Staddon JM, Saitou M, et al. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci*. 1997;110 (Pt 14)(14):1603-1613. doi:10.1242/JCS.110.14.1603

9. Lampugnani MG, Corada M, Caveda L, et al. The Molecular Organization of Endothelial Cell to Cell Junctions: Differential Association of Plakoglobin,/3-catenin, and ot-catenin with Vascular

Endothelial Cadherin (VE-cadherin). Accessed June 17, 2024. http://rupress.org/jcb/article-pdf/129/1/203/1478477/203.pdf

10. Mckenzie JAG, Ridley AJ. Roles of Rho/ROCK and MLCK in TNF-a-Induced Changes in Endothelial Morphology and Permeability. *J Cell Physiol*. 2007;213:221-228. doi:10.1002/jcp.21114

11. Hoelzle MK, Svitkina T. A Highlights from MBoC Selection: The cytoskeletal mechanisms of cell–cell junction formation in endothelial cells. *Mol Biol Cell*. 2012;23(2):310. doi:10.1091/MBC.E11-08-0719

12. Taha AA, Taha M, Seebach J, Schnittler HJ. ARP2/3-mediated junction-associated lamellipodia control VE-cadherin–based cell junction dynamics and maintain monolayer integrity. *Mol Biol Cell*. 2014;25(2):245. doi:10.1091/MBC.E13-07-0404

13. Seebach J, Donnert G, Kronstein R, et al. Regulation of endothelial barrier function during flow-induced conversion to an arterial phenotype. Published online 2007. doi:10.1016/j.cardiores.2007.04.017

14. van der Stoel MM, Kotini MP, Schoon RM, Affolter M, Belting HG, Huveneers S. Vinculin strengthens the endothelial barrier during vascular development. *Vascular Biology*. 2022;5(1). doi:10.1530/vb-22-0012

15. Lee MJ, Thangada S, Claffey KP, et al. Vascular Endothelial Cell Adherens Junction Assembly and Morphogenesis Induced by Sphingosine-1-Phosphate. *Cell*. 1999;99(3):301-312. doi:10.1016/S0092-8674(00)81661-X

16. Fernández-Martín L, Marcos-Ramiro B, Bigarella CL, et al. Crosstalk between reticular adherens junctions and platelet endothelial cell adhesion molecule-1 regulates endothelial barrier function. *Arterioscler Thromb Vasc Biol*. 2012;32(8):90-102. doi:10.1161/ATVBAHA.112.252080

17. Daniel AE, Timmerman I, Kovacevic I, et al. Plasminogen Activator Inhibitor-1 Controls Vascular Integrity by Regulating VE-Cadherin Trafficking. Published online 2015. doi:10.1371/journal.pone.0145684

18. Noda K, Zhang J, Fukuhara S, Kunimoto S, Yoshimura M, Mochizuki N. Vascular Endothelial-Cadherin Stabilizes at Cell-Cell Junctions by Anchoring to Circumferential Actin Bundles through-and-Catenins in Cyclic AMP-Epac-Rap1 Signal-activated Endothelial Cells. *Mol Biol Cell*. 2010;21:584-596. doi:10.1091/mbc.E09

19. Yuan D, He P. Vascular remodeling alters adhesion protein and cytoskeleton reactions to inflammatory stimuli resulting in enhanced permeability increases in rat venules. *J Appl Physiol*. 2012;113:1110-1120. doi:10.1152/japplphysiol.00102.2012.-Vascular

20. Oldenburg J, Van Der Krogt G, Twiss F, et al. VASP, zyxin and TES are tension-dependent members of Focal Adherens Junctions independent of the α-catenin-vinculin module. *Sci Rep*. 2015;5. doi:10.1038/srep17225

21. Huveneers S, Oldenburg J, Spanjaard E, et al. Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. *Journal of Cell Biology*. 2012;196(5):641-652. doi:10.1083/jcb.201108120

22. A L, C A, F G, K G, P C. Tight junctions at the blood-brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS*. 2012;9:23.

23. Dong X. Current strategies for brain drug delivery. *Theranostics*. 2018;8(6):1481-1493. doi:10.7150/thno.21254

24. Pardridge WM. The blood-brain barrier: Bottleneck in brain drug development. *Neurotherapeutics*. 2005;2(1):3-14. doi:10.1007/bf03206638

25. Nyqvist D, Giampietro C, Dejana E. Deciphering the functional role of endothelial junctions by using in vivo models. *EMBO Rep*. 2008;9(8):742-747. doi:10.1038/embor.2008.123

26. Dejana E, Orsenigo F. Endothelial adherens junctions at a glance. *J Cell Sci*. 2013;126:2545-2549. doi:10.1242/jcs.124529

27. Lampugnani MG. Endothelial cell-to-cell junctions: Adhesion and signaling in physiology and pathology. *Cold Spring Harb Perspect Med*. 2012;2(10):1-14. doi:10.1101/cshperspect.a006528

28. Dejana E, Orsenigo F, Molendini C, Baluk P, McDonald DM. Organization and signaling of endothelial cell-to-cell junctions in various regions of the blood and lymphatic vascular trees. *Cell Tissue Res*. 2009;335(1):17-25. doi:10.1007/s00441-008-0694-5

29. Aird WC. Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med*. 2012;2(1):1-13. doi:10.1101/cshperspect.a006429

30. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res*. 2007;100(2):158-173. doi:10.1161/01.RES.0000255691.76142.4a

31. Aird WC. Phenotypic Heterogeneity of the Endothelium II. Representative Vascular Beds. *Circ Res*. 2007;100(2):174-190. doi:10.1161/01.RES.0000255690.03436.ae

32. Reglero-Real N, Colom B, Bodkin JV, Nourshargh S. Endothelial Cell Junctional Adhesion Molecules: Role and Regulation of Expression in Inflammation. *Arterioscler Thromb Vasc Biol*. 2016;36(10):2048-2057. doi:10.1161/ATVBAHA.116.307610

33. Komarova, Y., Kruse, K., Mehta, D., and Malik A. Protein interactions at endothelial junctions and signaling mechanisms regulating endothelial permeability. *Circ Res*. 2017;120(1). doi:10.1016/j.physbeh.2017.03.040

34. Park-Windhol C, D'Amore PA. Disorders of Vascular Permeability. *Annual Review of Pathology: Mechanisms of Disease*. 2016;11:251-281. doi:10.1146/annurev-pathol-012615-044506

35. Komarova Y, Malik AB. Regulation of Endothelial Permeability via Paracellular and Transcellular Transport Pathways. *Annu Rev Physiol*. 2010;72(1):463-493. doi:10.1146/annurev-physiol-021909-135833

36. Wettschureck N, Strilic B, Offermanns S. Passing the Vascular Barrier: Endothelial Signaling Processes Controlling Extravasation. *Physiol Rev*. 2019;99(3):1467-1525. doi:10.1152/physrev.00037.2018

37. Mehta D, Malik AB. Signaling Mechanisms Regulating Endothelial Permeability. *Physiol Rev*. 2006;86:279-367. doi:10.1152/physrev.00012.2005.

38. Muller WA. Mechanisms of Leukocyte Transendothelial Migration. *Annu Rev Pathol*. 2011;6:323-344. doi:10.1146/annurev-pathol-011110-130224.Mechanisms

39. Aird WC. Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med*. 2012;2(1):1-13. doi:10.1101/cshperspect.a006429

40. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res*. 2007;100(2):158-173. doi:10.1161/01.RES.0000255691.76142.4a

41. Aird WC. Phenotypic Heterogeneity of the Endothelium II. Representative Vascular Beds. *Circ Res*. 2007;100(2):174-190. doi:10.1161/01.RES.0000255690.03436.ae

42. Förster C. Tight junctions and the modulation of barrier function in disease. *Histochem Cell Biol*. 2008;130(1):55-70. doi:10.1007/s00418-008-0424-9

43. Claude P. *Morphological Factors Influencing Transepithelial Permeability" A Model for the Resistance of the Zonula Occludens*. Vol 39.; 1978.

44. Clayburgh DR, Shen L, Turner JR. A porous defense: The leaky epithelial barrier in intestinal disease. *Laboratory Investigation*. 2004;84(3):282-291. doi:10.1038/labinvest.3700050

45. Gumbiner B. Structure, biochemistry, and assembly of epithelial tight junctions. *American Journal of Physiology*. Published online 1987:C749-C758.

46. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. *Cold Spring Harb Perspect Biol*. 2009;1(2). doi:10.1101/cshperspect.a002584

47. Morita K, Sasaki H, Furuse M, Tsukita S. *Endothelial Claudin: Claudin-5/TMVCF Constitutes Tight Junction Strands in Endothelial Cells*. Vol 147.; 1999. http://www.jcb.org

48. Nitta T, Hata M, Gotoh S, et al. Size-selective loosening of the blood-brain barrier in claudin-5–deficient mice. *Journal of Cell Biology*. 2003;161(3):653-660. doi:10.1083/JCB.200302070

49. Corada M, Mariotti M, Thurston G, et al. Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo (endotheliumpermeabilityadherens junction). *Medical Sciences*. 1999;96:9815-9820. Accessed May 15, 2024. www.pnas.org.

50. Cattelino A, Liebner S, Gallini R, et al. The conditional inactivation of the-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility. *J Cell Biol*. 2003;162(6):1111-1122. doi:10.1083/jcb.200212157

51. Lampugnani MG, Dejana E. *Adherens Junctions in Endothelial Cells Regulate Vessel Maintenance and Angiogenesis*. Vol 120.; 2007.

52. Leach L, Babawale MO, Anderson M, Lammiman M. Vasculogenesis, angiogenesis and the molecular organisation of endothelial junctions in the early human placenta. *J Vasc Res*. 2002;39(3):246-259. doi:10.1159/000063690

53. Dye JF, Leach L, Clark P, Firth JA. Cyclic AMP and acidic fibroblast growth factor have opposing effects on tight and adherens junctions in microvascular endothelial cells in vitro. *Microvasc Res*. 2001;62(2):94-113. doi:10.1006/mvre.2001.2333

54. Leach L. The phenotype of the human materno-fetal endothelial barrier: Molecular occupancy of paracellular junctions dictate permeability and angiogenic plasticity. *J Anat*. 2002;200(6):599-606. doi:10.1046/j.1469-7580.2002.00062.x

55. Neto F, Klaus-Bergmann A, Ong YT, et al. YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development. *Elife*. 2018;7:1-30. doi:10.7554/eLife.31037

56. Maeso-Alonso L, Alonso-Olivares H, Martínez-García N, et al. p73 is required for vessel integrity controlling endothelial junctional dynamics through Angiomotin. 2022;79:535. doi:10.1007/s00018-022-04560-3

57. Klomp JE, Shaaya M, Matsche J, Chew TL, Malik AB, Karginov Correspondence A V. Time-Variant SRC Kinase Activation Determines Endothelial Permeability Response. *Cell Chem Biol*. 2019;26:1081-1094.e6. doi:10.1016/j.chembiol.2019.04.007

58. Wilson CW, Ye W. Regulation of vascular endothelial junction stability and remodeling through Rap1-Rasip1 signaling. *Cell Adh Migr*. 2014;8(2):76-83. doi:10.4161/cam.28115

59. Li B, Zhao WD, Tan ZM, Fang WG, Zhu L, Chen YH. Involvement of Rho/ROCK signalling in small cell lung cancer migration through human brain microvascular endothelial cells. *FEBS Lett*. 2006;580(17):4252-4260. doi:10.1016/j.febslet.2006.06.056

60. Stroka KM, Vaitkus JA, Aranda-espinoza H. Endothelial cells undergo morphological , biomechanical , and dynamic changes in response to tumor necrosis factor- a. Published online 2012:939-947. doi:10.1007/s00249-012-0851-3

61. Millán J, Cain RJ, Reglero-Real N, et al. *Adherens Junctions Connect Stress Fibres between Adjacent Endothelial Cells*.; 2010. doi:10.1186/1741-7007-8-11

62. Onken MD, Mooren OL, Mukherjee S, Shahan ST, Li J, Cooper JA. Endothelial monolayers and transendothelial migration depend on mechanical properties of the substrate. *Cytoskeleton*. 2014;71(12):695-706. doi:10.1002/CM.21203

63. Inglut CT, Gray K, Vig S, et al. Photodynamic Priming Modulates Endothelial Cell–Cell Junction Phenotype for Light-activated Remote Control of Drug Delivery. *IEEE Journal of Selected Topics in Quantum Electronics*. 2020;27(4):1-1. doi:10.1109/jstqe.2020.3024014

64. Gray KM, Jung JW, Inglut CT, Huang HC, Stroka KM. Quantitatively relating brain endothelial cell-cell junction phenotype to global and local barrier properties under varied culture conditions via the Junction Analyzer Program. *Fluids Barriers CNS*. 2020;17:16. doi:10.1186/s12987-020-0177-y

65. Gray KM, Katz DB, Brown EG, Stroka KM. Quantitative Phenotyping of Cell–Cell Junctions to Evaluate ZO-1 Presentation in Brain Endothelial Cells. *Ann Biomed Eng*. 2019;47(7):1675-1687. doi:10.1007/s10439-019-02266-5

66. Pranda MA, Gray KM, DeCastro AJL, Dawson GM, Jung JW, Stroka KM. Tumor Cell Mechanosensing During Incorporation into the Brain Microvascular Endothelium. *Cell Mol Bioeng*. 2019;12(5):455-480. doi:10.1007/s12195-019-00591-2

67. Andrews AM, Ramirez SH, Lutton EM, Merkel SF, Razmpour R. Mechanical Injury Induces Brain Endothelial-Derived Microvesicle Release: Implications for Cerebral Vascular Injury during Traumatic Brain Injury. *Frontiers in Cellular Neuroscience | www.frontiersin.org*. 2016;10:43. doi:10.3389/fncel.2016.00043

68. Huang J, Ge S, Luo D, et al. The endothelium permeability after bioresorbable scaffolds implantation caused by the heterogeneous expression of tight junction proteins. *Mater Today Bio*. Published online 2022. doi:10.1016/j.mtbio.2022.100410

69. Garcia-Flores AE, Gross CM, Zemskov EA, et al. Loss of SOX18/CLAUDIN5 disrupts the pulmonary endothelial barrier in ventilator-induced lung injury. *Front Physiol*. 2022;13(December):1-13. doi:10.3389/fphys.2022.1066515

70. Collins NT, Cummins PM, Colgan OC, et al. Cyclic strain-mediated regulation of vascular endothelial occludin and ZO-1: Influence on intercellular tight junction assembly and function. *Arterioscler Thromb Vasc Biol*. 2006;26(1):62-68. doi:10.1161/01.ATV.0000194097.92824.b3

71. Brezovjakova H, Tomlinson C, Mohd-Naim N, et al. Junction mapper is a novel computer vision tool to decipher cell-cell contact phenotypes. *Elife*. 2019;8. doi:10.7554/eLife.45413

72. Huynh J, Nishimura N, Rana K, et al. Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration. *Sci Transl Med*. 2011;3(112):1-10. doi:10.1126/scitranslmed.3002761

73. Held C, Palmisano R, Häberle L, Hensel M, Wittenberg T. Comparison of parameter-adapted segmentation methods for fluorescence micrographs. *Cytometry Part A*. 2011;79 A(11):933-945. doi:10.1002/cyto.a.21122

74. Motallebnejad P, Rajesh V V, Azarin SM. Evaluating the Role of IL-1b in Transmigration of Triple Negative Breast Cancer Cells Across the Brain Endothelium. doi:10.1007/s12195-021-00710-y

75. Puhlmann M, Weinreich DM, Farma JM, Carroll NM, Turner EM, Alexander HR. Interleukin-1β induced vascular permeability is dependent on induction of endothelial tissue factor (TF) activity. *J Transl Med*. 2005;3:1-7. doi:10.1186/1479-5876-3-37

76. Burns AR, Walker DC, Evelyn S, et al. Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. . *J Immunol*. 1997;159(6):2893-2903. doi:https://doi.org/10.4049/jimmunol.159.6.2893 Related

77. Dias MC, Quesada AO, Soldati S, et al. Brain endothelial tricellular junctions as novel sites for T cell diapedesis across the blood-brain barrier. *J Cell Sci*. 2021;134(8). doi:10.1242/jcs.253880

78. Burns AR, Walker DC, Evelyn S, et al. Neutrophil transendothelial migration is independent of tight junctions and occurs preferentially at tricellular corners. . *J Immunol*. 1997;159(6):2893-2903. doi:https://doi.org/10.4049/jimmunol.159.6.2893 Related

79. Burns AR, Bowden RA, Macdonell SD, et al. Analysis of tight junctions during neutrophil transendothelial migration. *J Cell Sci*. 2000;113:45-57.

80. Walker DC, MacKenzie A, Hosford S. The Structure of the Tricellular Region of Endothelial Tight Junctions of Pulmonary Capillaries Analyzed by Freeze-Fracture. *Microvasc Res*. 1994;48(3):259-281. doi:https://doi.org/10.1006/mvre.1994.1054

81. Jafri RZ, Ali A, Messonnier NE, et al. Global epidemiology of invasive meningococcal disease. *Popul Health Metr*. 2013;11(1):1-9. doi:10.1186/1478-7954-11-17/FIGURES/2

82. Coureuil M, Lécuyer H, Bourdoulous S, Nassif X. A journey into the brain: insight into how bacterial pathogens cross blood–brain barriers. *Nature Reviews Microbiology 2017 15:3*. 2017;15(3):149-159. doi:10.1038/nrmicro.2016.178

83. Endres LM, Jungblut M, Divyapicigil M, et al. Development of a multicellular in vitro model of the meningeal blood-CSF barrier to study Neisseria meningitidis infection. doi:10.1186/s12987-022-00379-z

84. Hoefer IE, Den Adel B, Daemen MJAP. Biomechanical factors as triggers of vascular growth. *Cardiovasc Res*. 2013;99(2):276-283. doi:10.1093/cvr/cvt089

85. Gray KM, Stroka KM. Vascular endothelial cell mechanosensing: New insights gained from biomimetic microfluidic models. *Semin Cell Dev Biol*. Published online 2017. doi:10.1016/j.semcdb.2017.06.002

86. Yap AS, Duszyc K, Viasnoff V. Mechanosensing and mechanotransduction at cell –cell junctions. *Cold Spring Harb Perspect Biol*. 2018;10(8). doi:10.1101/cshperspect.a028761

87. Lagendijk AK, Gomez GA, Baek S, et al. Live imaging molecular changes in junctional tension upon VE-cadherin in zebrafish. doi:10.1038/s41467-017-01325-6

88. Conway DE, Breckenridge MT, Hinde E, Gratton E, Chen CS, Schwartz MA. Fluid shear stress on endothelial cells modulates mechanical tension across VE-cadherin and PECAM-1. *Current Biology*. 2013;23(11):1024-1030. doi:10.1016/j.cub.2013.04.049

89. Rosson D, O'brien TG, Kampherstein JA, et al. Protein Kinase C-Î± Activity Modulates Transepithelial Permeability and Cell Junctions in the LLC-PK1 Epithelial Cell Line*. *J Biol Chem*. 1997;272:14950-14953. doi:10.1074/jbc.272.23.14950

90. Balda MS, Gonzfilez-Mariscal L, Contreras RG, et al. Membrane Biology Assembly and Sealing of Tight Junctions: Possible Participation of G-proteins, Phospholipase C, Protein Kinase C and Calmodulin. *J Membrane Biol*. 1991;122:002226319100093.

91. de Graaf MNS, Vivas A, Kasi DG, et al. Multiplexed fluidic circuit board for controlled perfusion of 3D blood vessels-on-a-chip. *Lab Chip*. 2022;23(1):168. doi:10.1039/D2LC00686C

92. Urbano RL, Furia C, Basehore S, Clyne AM. Stiff Substrates Increase Inflammation-Induced Endothelial Monolayer Tension and Permeability. *Biophys J*. 2017;113(3):645-655. doi:10.1016/j.bpj.2017.06.033

93. Yan L, Dwiggins CW, Moriarty RA, et al. Matrix stiffness regulates the tight junction phenotypes and local barrier properties in tricellular regions in an iPSC-derived BBB model. *Acta Biomater*. 2023;167:109-120. doi:10.1016/j.actbio.2023.06.003

94. Bordeleau F, Mason BN, Lollis EM, et al. Matrix stiffening promotes a tumor vasculature phenotype. *Proc Natl Acad Sci U S A*. 2017;114(3):492-497. doi:10.1073/pnas.1613855114

95. Bastounis EE, Yeh YT, Theriot JA. Subendothelial stiffness alters endothelial cell traction force generation while exerting a minimal effect on the transcriptome. *Sci Rep*. 2019;9(1):1-16. doi:10.1038/s41598-019-54336-2

96. Stroka KM, Aranda-Espinoza H. Endothelial cell substrate stiffness influences neutrophil transmigration via myosin light chain kinase-dependent cell contraction. *Blood*. 2011;118(6):1632-1640. doi:10.1182/blood-2010-11-321125

97. Sun C, Wu MH, Yuan SY. Nonmuscle myosin light-chain kinase deficiency attenuates atherosclerosis in apolipoprotein e-deficient mice via reduced endothelial barrier dysfunction and monocyte migration. *Circulation*. 2011;124(1):48-57. doi:10.1161/CIRCULATIONAHA.110.988915

98. Burridge K, Wennerberg K. Rho and Rac Take Center Stage. *Cell*. 2004;116(2):167-179. doi:10.1016/S0092-8674(04)00003-0

99. Kazakova OA, Khapchaev AY, Shirinsky VP. MLCK and ROCK mutualism in endothelial barrier dysfunction: Joint Work of MLCK and ROCK in Endothelium. *Biochimie*. 2020;168:83-91. doi:10.1016/j.biochi.2019.10.010

100. Mammoto A, Mammoto T, Kanapathipillai M, et al. Control of lung vascular permeability and endotoxin-induced pulmonary oedema by changes in extracellular matrix mechanics. *Nat Commun*. 2013;4. doi:10.1038/ncomms2774

101. Mouw JK, Ou G, Weaver VM. Extracellular matrix assembly: a multiscale deconstruction. *Nat Rev Mol Cell Biol*. 2014;15(12):771. doi:10.1038/NRM3902

102. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech*. 2011;4(2):165-178. doi:10.1242/DMM.004077

103. Carey SP, D'Alfonso TM, Shin SJ, Reinhart-King CA. Mechanobiology of tumor invasion: Engineering meets oncology. *Crit Rev Oncol Hematol*. 2012;83(2):170-183. doi:10.1016/j.critrevonc.2011.11.005

104. Silvani G, Romanov V, Cox CD, Martinac B. Biomechanical Characterization of Endothelial Cells Exposed to Shear Stress Using Acoustic Force Spectroscopy. *Front Bioeng Biotechnol*. 2021;9:612151. doi:10.3389/FBIOE.2021.612151/BIBTEX

105. Garcia-Polite F, Martorell J, Del Rey-Puech P, et al. Pulsatility and high shear stress deteriorate barrier phenotype in brain microvascular endothelium. *Journal of Cerebral Blood Flow and Metabolism*. 2017;37(7):2614-2625. doi:10.1177/0271678X16672482

106. Molins B, Mora A, Romero-Vázquez S, et al. Shear stress modulates inner blood retinal barrier phenotype. *Exp Eye Res*. 2019;187(January):107751. doi:10.1016/j.exer.2019.107751

107. Ghim M, Yang S wook, David KRZ, Eustaquio J, Warboys CM, Weinberg PD. NO Synthesis but Not Apoptosis , Mitosis or Inflammation Can Explain Correlations between Flow Directionality and Paracellular Permeability of Cultured Endothelium. Published online 2022.

108. Staughton TJ, Lever MJ, Weinberg PD. Effect of altered flow on the pattern of permeability around rabbit aortic branches. *Am J Physiol Heart Circ Physiol*. 2001;281(1 50-1):53-59. doi:10.1152/ajpheart.2001.281.1.h53

109. Miao H, Hu YL, Shiu YT, et al. Effects of flow patterns on the localization and expression of VE-cadherin at vascular endothelial cell junctions: In vivo and in vitro investigations. *J Vasc Res*. 2005;42(1):77-89. doi:10.1159/000083094

110. Bouhrira N, DeOre BJ, Galie PA, Peter Galie CA. Implementation and characterization of a physiologically relevant flow waveform in a 3D microfluidic model of the blood-brain barrier. *Biotechnol Bioeng*. 2021;118:2411-2421. doi:10.1002/bit.27719

111. Wong JF, Simmons CA. Microfluidic assay for the on-chip electrochemical measurement of cell monolayer permeability. *Lab Chip*. 2019;19(6):1060-1070. doi:10.1039/C8LC01321G

112. Dubrovskyi O, Birukova AA, Birukov KG. Measurement of local permeability at subcellular level in cell models of agonist-and ventilator-induced lung injury. *Laboratory Investigation*. 2013;93(2):254-263. doi:10.1038/labinvest.2012.159

113. Gao F, Sun H, Li X, He P. Leveraging avidin-biotin interaction to quantify permeability property of microvessels-on-a-chip networks. *Am J Physiol Heart Circ Physiol*. 2022;322(1):H71-H86. doi:10.1152/AJPHEART.00478.2021

114. Shirakura K, Baluk P, Nottebaum AF, et al. Shear stress control of vascular leaks and atheromas through Tie2 activation by VE-PTP sequestration. *EMBO Mol Med*. 2023;15(4). doi:10.15252/emmm.202216128

115. Zhao S, Suciu A, Ziegler T, et al. Synergistic effects of fluid shear stress and cyclic circumferential stretch on vascular endothelial cell morphology and cytoskeleton. *Arterioscler Thromb Vasc Biol*. 1995;15(10):1781-1786. doi:10.1161/01.ATV.15.10.1781

116. Meza D, Musmacker B, Steadman E, Stransky T, Rubenstein DA, Yin W. Endothelial Cell Biomechanical Responses are Dependent on Both Fluid Shear Stress and Tensile Strain. *Cell Mol Bioeng*. 2019;12(4):311-325. doi:10.1007/s12195-019-00585-0

117. Breen LT, McHugh PE, Murphy BP. Multi-axial mechanical stimulation of HUVECs demonstrates that combined loading is not equivalent to the superposition of individual wall shear stress and tensile hoop stress components. *J Biomech Eng*. 2009;131(8):1-10. doi:10.1115/1.3127248