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Sensing of shear stress in vascular endothelial cells: from physiology to pathology

Eulashini Chuntharpursat-Bon[1], Jovana Serbanovic-Canic[2], Mabruka Alfaidi[3], Elizabeth A. V. Jones[4], Caroline Cheng[5], Stephen White[6], Tzung Hsiai[7], Hanjoong Jo[8], Nicolas Baeyens[9], Yun Fang[10], Eno E. Ebong[11], John S Reader[12], Ellie Tzima[12], Suowen Xu[13], Paul C Evans[14]

1. School of Medicine, University of Leeds, LS2 9JT, UK
2. School of Medicine and Population Health, University of Sheffield, Sheffield, UK
3. Department of Cellular and Integrative Physiology, Center for Heart and Vascular Research, University of Nebraska Medical Center, Omaha, NE, USA.
4. Centre for Molecular and Vascular Biology, Department of Cardiovascular Sciences, KU Leuven, 3000 Leuven, Belgium; CARIM – Research institute for Cardiovascular Diseases, Maastricht University, 6200 MD Maastricht, The Netherlands
5. University Medical Centre, Utrecht
6. Newcastle University, UK
7. Department of Bioengineering, UCLA, Los Angeles, CA, 90095, USA.
8. Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology, Atlanta, GA, USA
9. Laboratoire de Physiologie et Pharmacologie, Faculté de Médecine, Université libre de Bruxelles, Brussels, Belgium.
10. Section of Pulmonary and Critical Care, Department of Medicine, University of Chicago, Chicago, IL, USA.
11. Departments of Chemical Engineering and Bioengineering, Northeastern University, Boston, MA, USA, and Department of Neuroscience, Albert Einstein College of Medicine, New York, NY, USA
12. Radcliffe Department of Medicine, University of Oxford, OX3 9DU
13. Department of Endocrinology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, 230000, China. sxu1984@ustc.edu.cn.
14. William Harvey Research Institute, Queen Mary University of London, London, UK (paul.evans@qmul.ac.uk)

Abstract

Sensing of mechanical force is crucial in regulating vascular homeostasis, physiology and disease. Endothelial cells line the lumens of blood vessels and are constantly exposed to flowing blood. This generates mechanical shear stress, which is instrumental in modulating endothelial cell behaviour. Mechanosensitive proteins, including ion channels, G protein-coupled receptors (GPCR) and other cell surface receptors, adhesion molecules, integrins, primary cilia, cytoskeletal elements and the glycocalyx, transduce mechanical stimuli into biochemical signals that are essential for maintaining vascular integrity, responding to inflammatory stimuli and facilitating angiogenesis and arteriogenesis. Disruption in shear stress sensing can lead to pathological conditions such as atherosclerosis or vascular anomalies. This Review article provides an integrated overview of the current knowledge on endothelial shear stress sensing and highlights key unanswered questions that will shape future research in vascular biology and disease.

Introduction

Endothelial cells (ECs), which line the inner surface of blood vessels, are exquisitely sensitive to flowing blood, which generates hemodynamic forces such as shear stress (Dewey et al., 1981) (Fig. 1). ECs possess an array of specialised mechanosensors that sense and respond to shear stress, enabling them to transduce mechanical stimuli into intracellular biochemical signals. This process, termed mechanotransduction, plays a crucial role in vascular homeostasis, development and pathologies (Dewey et al., 1981). Shear stress is central to an important homeostatic mechanism that operates in arterial remodelling. When blood flow increases, vessel wall shear stress rises, triggering outward (expansive, larger diameter) remodelling of the vessel. The resulting increase in luminal calibre reduces flow velocity, allowing shear stress to return toward its baseline level. Conversely, reduced blood flow leads to reduced shear stress and inward remodelling (smaller diameter), which increases flow velocity and restores shear stress toward baseline. This regulated baseline level is referred to as the shear stress set point (Baeyens et al., 2015).

Dysregulation of vascular mechanotransduction has been implicated in pathological conditions such as hypertension, vascular anomalies and atherosclerosis (Albarrán-Juárez et al., 2018; Goel et al., 2008; Harry et al., 2008; Stevens et al., 2008; Mehta et al., 2020). Physiological shear stress, which is typically uniform and unidirectional (laminar) and relatively high magnitude, reduces inflammation and vascular permeability and is protective against atherosclerosis (atheroprotective). Arterial regions exposed to disturbed flow, such as branch points or bends, are characterised by lower magnitude and/or oscillatory (multidirectional) shear stress, which, in contrast to physiological shear stress, is atherogenic (Fig. 1). Endothelial mechanosensors can differentially sense and respond to physiological *versus* disturbed flow and different levels of shear stress, activating specific downstream signalling pathways.

This Review explores the diverse and complex array of mechanosensors found in ECs, including ion channels, G protein-coupled receptors (GPCRs) and other cell surface receptors, adhesion molecules, primary cilia and components of the EC glycocalyx (Fig. 2). These structures and molecules can serve as ‘apex’ mechanosensors – primary transducers of mechanical force – or as mechanoamplifiers that enhance downstream signalling (Lim and Harraz, 2024; Cox et al., 2024). We give particular

emphasis to ion channels, such as PIEZO1 and transient receptor potential cation channel subfamily V member 4 (TRPV4), as well as adhesion molecules, such as platelet endothelial cell adhesion molecule (PECAM-1) and vascular endothelial (VE)-cadherin, and recent research highlighting their potential interaction. We also overview emerging data that point to biophysical properties of membranes, such as fluidity, as well as flow-sensitive structures like primary cilia and the glycocalyx as key components in mechanosensory function. Finally, we discuss how physical activity and exercise can beneficially modulate these mechanosensory pathways through the induction of shear stress, offering promising therapeutic implications for cardiovascular health.

Ion channels

Mechanoelectrical transduction – the process of converting mechanical stimuli into intracellular electrochemical signals – is a fundamental, universally evolutionarily conserved mechanism, driven by mechanosensitive ion channels (Jin et al., 2020; Poole, 2022). These ion channels, which act as force sensors, form pores in cell membranes. Mechanical forces serve as gating signals that activate the channel, allowing ions to pass through the membrane (Richardson et al., 2022). The gating of mechanosensitive channels is directly influenced by mechanical inputs, resulting in rapid and temporary signalling, making such channels apex mechanotransducers that initiate mechanosignalling pathways.

PIEZO1

PIEZO1 is an apex mechanosensor in ECs: PIEZO1-mediated ion entry can be observed upon mechanical stimulation in a reconstituted system lacking other cellular components (Coste et al., 2010; Arnadóttir and Chalfie, 2010 Syeda et al., 2016). It is a nonselective cation channel that is more permeable to Ca^{2+} than Na^{+} and its activation, whether mechanical (e.g., via shear stress) or chemical (e.g., via the activator Yoda1), leads to ionic currents and intracellular Ca^{2+} signals in ECs (Harraz et al., 2022; Li et al., 2014; Ranade et al., 2014; Wang et al., 2016). PIEZO1 is essential for flow-mediated EC alignment during vascular development, in which shear stress induces ECs to orient in the direction of flow (Li et al., 2014; Ranade et al., 2014). The mechanism underlying this phenomenon requires PIEZO1-induced Ca^{2+} transients that activate enzymes necessary for EC alignment, including the Ca^{2+}-dependent

protease calpain (Li et al., 2014), which is vital for stress fibre orientation, angiogenesis and cerebrovascular patterning (Kang et al., 2018; Liu et al., 2020; Ranade et al., 2014). The mechanism of PIEZO1 channel opening has been found to involve alterations in membrane fluidity (Buyan et al., 2020) and lipid interactions (Smith et al., 2025).

PIEZO1 also influences control of vascular diameter. It can mediate both vasodilation and vasoconstriction depending on specific vascular contexts, though further research is needed to resolve discrepancies (Rode et al., 2017; Wang et al., 2016). By promoting nitric oxide (NO) synthesis through Ca^{2+}-dependent activation of endothelial NO synthase (eNOS), PIEZO1 activation leads to vasodilation. Additionally, PIEZO1 facilitates ATP release, which activates purinergic Gq/11-protein coupled receptors (Gq/11-PCRs) to enhance NO production (Albarrán-Juárez et al., 2018). Although PIEZO1 has been linked to regulation of blood pressure, some findings suggest its expression and role can vary across vascular beds (Chuntharpursat-Bon et al., 2023a; Endesh et al., 2023; Evans et al., 2018; John et al., 2018; Lhomme et al., 2019; Wang et al., 2016). Genome-wide investigation of data in the UK Biobank has revealed genetic variants of PIEZO1 associated with various vascular diseases, including lymphatic malformations, varicose veins and arterial venous malformations (Cheng et al., 2025).

PIEZO1 plays a key role in the spatial localisation of atherosclerosis, a lipid-driven disease of arteries that develops preferentially at arterial regions exposed to disturbed flow characterised by low shear stress (Albarrán-Juárez et al., 2018). Notably, PIEZO1 can sense both physiological shear stress, which is atheroprotective, and oscillatory and low shear stress, which are atheroprone. However, under low shear stress conditions, PIEZO1 signalling activates specific downstream pathways associated with atherosclerosis, including the G_q/G_{11} pathway (Albarrán-Juárez et al., 2018) and in neutrophils leads to the formation of neutrophil extracellular traps (NETs) (Zhu et al., 2024). PIEZO1 also activates Notch signalling, which is a central regulator of vascular development, homeostasis and disease (Qin et al., 2016) (Souilhol et al., 2022). PIEZO1-dependent activation of the cell surface metalloproteases ADAM10 and ADAM17 leads to cleavage of Notch, which releases the Notch intracellular domain, resulting in transcriptional activation of Notch target genes (Caolo et al., 2020). In

arteries, physiological shear stress drives protection from atherosclerosis via NOTCH1 (Qin et al., 2016), whereas low shear stress promotes disease initiation at branches and bends via NOTCH4 (Souilhol et al., 2022). In summary, PIEZO1 is a crucial mechanosensor in ECs, with its function and expression varying depending on vascular bed type and size.

TRPV4

TRPV4, a nonselective transient receptor potential (TRP) cation channel with higher permeability to Ca^{2+}, was first identified in ECs over 20 years ago and is present in various vascular beds, such as mesenteric arteries, pulmonary arteries and brain capillaries (Earley and Brayden, 2015; Hartmannsgruber et al., 2007; Köhler et al., 2006; Mendoza et al., 2009; Watanabe et al., 2002). TRPV4 contributes to vascular functions like tone regulation, EC orientation, angiogenesis and permeability (Earley and Brayden, 2015; White et al., 2016). Although TRPV4 is sensitive to mechanical forces, whether it is a true mechanosensor or a mechanoamplifier has been debated (Nikolaev et al., 2019; Swain and Liddle, 2020b). Instead of directly sensing force, TRPV4 might be activated by substances like arachidonic acid and epoxyeicosatrienoic acids that are released during shear stress responses (Köhler et al., 2006; Loot et al., 2008; Watanabe et al., 2003). PIEZO1 activation has been shown to enhance TRPV4 activity, suggesting PIEZO1 acts as the apex mechanosensor, with TRPV4 amplifying Ca^{2+} signalling downstream (Swain and Liddle, 2020a).

- TRPV4 activation in ECs leads to vasodilation via both NO release and membrane hyperpolarization, and it is possible that these pathways are activated simultaneously to act together (Hartmannsgruber et al., 2007; Köhler et al., 2006; Mendoza et al., 2009; Sonkusare et al., 2012). TRPV4-mediated Ca^{2+} signals activate eNOS to promote NO production or stimulate Ca^{2+}-activated K^+ channels to induce hyperpolarization and genetic deletion of TRPV4 reduces both Ca^{2+} responses and NO release (McFarland et al., 2020). TRPV4 is also activated by GqPCR signalling, which triggers downstream pathways like protein kinase C (PKC) and inositol triphosphate receptor (IP3R) signalling, further amplifying Ca^{2+} signalling (Harraz et al., 2018; Heathcote et al., 2019; Sonkusare et al., 2014). Furthermore, TRPV4 interacts with proteins like caveolin-1, which is essential for EC signalling, vasodilation (Rath et al., 2012; Saliez et al., 2008) and control of vascular inflammation

(PMID: 2179550). Recent research has shown that in ECs under flow, TRPV4 accumulates in specialized caveolin-1-enriched membrane domains, consolidating the role of flow in EC activation and anti-inflammatory contribution (Hong et al., 2024).

Kir2.1

One of the earliest studies on endothelial mechanosensors identified a shear stress-sensitive K^+ channel in aortic ECs – specifically, an inward-rectifier K^+ (Kir) channel involved in flow-mediated hyperpolarisation and vascular tone regulation (Olesen et al., 1988). This discovery sparked interest in the role of Kir channels, particularly Kir2.1, in mechanosensation and blood flow control. Kir2.1, expressed in various ECs, is activated by hyperpolarisation and sensitive to extracellular K^+ levels (Ahn et al., 2016; Hibino et al., 2010; Longden et al., 2021; Zaritsky et al., 2000). Shear stress activates Kir2.1, leading to K^+ efflux, hyperpolarisation and potentially NO production (Ahn et al., 2016; Boriushkin et al., 2019; Hoger et al., 2002; Lieu et al., 2004). Kir2.1 is thought to be essential for activation of eNOS and Akt during flow (Ahn et al., 2016), though it might not be the apex mechanosensor. In contrast to PIEZO1, which is inherently mechanosensitive (Syeda et al., 2016), the role of KIR2.1 as a mechanosensor is dependent on cellular contexts, such as changes in membrane lipids or fluidity (Lieu et al., 2004; Jacobs et al., 1995; Boriushkin et al., 2019).

Epithelial sodium channel

Initially thought to be exclusive to epithelial cells, the epithelial sodium channel (ENaC) has since been found in ECs of various vascular tissues (Kusche-Vihrog et al., 2010; Sternak et al., 2018; Wang et al., 2009). ENaC in ECs is activated by physiological shear stress and its mechanosensitivity is dependent on interactions between the extracellular matrix, glycosylated α-ENaC residues and the endothelial glycocalyx, which aids in flow-mediated vasodilation (Knoepp et al., 2019; Sternak et al., 2018; Tarjus et al., 2017; Wang et al., 2009). Modulators like epoxyeicosatrienoic acids can inhibit ENaC, further emphasising its role in mechanosensing (Wang et al., 2009). Increased ENaC activity (gain-of-function) stiffens ECs and promotes vasoconstriction, which is often linked to hypertension (Jeggle et al., 2013; Zhang et al., 2022). This disrupts NO production by reducing L-arginine uptake or inhibiting the phosphoinositide 3-kinase (PI3K)-Akt pathway necessary for NO synthesis (Guo et al., 2016; Pérez et al., 2009). Thus, physiological levels of ENaC activity support shear

stress-mediated vasodilation, but excessive ENaC activity contributes to endothelial dysfunction (Jeggle et al., 2013). More research is needed to fully understand the complex role of ENaC in EC mechanosensing due to differences in ENaC activity levels in different vascular beds (Ashley et al., 2018; Zhang et al., 2022).

TREK-1

Two pore potassium (K2P) channels, including TRAAK, TREK-1 and TREK-2, are proposed endothelial mechanosensors involved in regulating vascular function (Gurney and Manoury, 2009). TREK-1, which has been extensively studied in ECs, responds to membrane tension by directly gating ion conduction, generating hyperpolarising currents (Brohawn, 2015; Brohawn et al., 2014; Honoré et al., 2006; Schmidpeter et al., 2023). It also contributes to NO-mediated vasodilation (Garry et al., 2007). TREK-1 is sensitive to osmotic pressure and ischemia; under ischemic conditions, it protects cerebrovascular ECs by promoting hyperpolarisation and vasodilation (Maingret et al., 1999; Patel et al., 1998; Rao et al., 1999). TREK-1 is modulated by GPCRs (Gi- and Gs- coupled receptors, specifically) and downstream signalling pathways, with phosphatidylinositol 4,5-bisphosphate (PIP2) signalling enhancing and cyclic AMP (cAMP) and protein kinase A (PKA) signalling inhibiting its activity (Brohawn et al., 2014; Chemin et al., 2004; Lesage et al., 2000; Mathie, 2006).

GPCRs

Activation of GPCRs typically occurs when an extracellular ligand binds to the receptor, triggering signalling cascades mediated by Ca^{2+}, cyclic nucleotides or β-arrestin. However, some GPCRs can induce ligand-independent mechanochemical signalling (Chachisvilis et al., 2006; Takada et al., 1997). This mechanosensitivity likely involves changes in membrane fluidity that allow GPCRs to activate without an agonist (Gudi et al., 1998; Yamamoto and Ando, 2013; Yamamoto and Ando, 2015). Helix 8, a conserved secondary structure in GPCRs following transmembrane segment 7, also contributes to GPCR mechanosensitivity by elongating in response to mechanical stimulation, leading to receptor activation (Erdogmus et al., 2019).

GPR68, a Gq/11-PCR found in ECs, promotes flow-induced vasodilation by increasing NO production and GPR68 deletion impairs this response, although studies show that flow activation can occur without helix 8 or G protein signalling (Erdogmus et al., 2019;

Ozkan et al., 2021). Other mechanosensitive GPCRs include the bradykinin 2 receptor (B2R), sphingosine-1-phosphate receptor 1 (S1PR1) and histamine H_1 receptor (H1R), all of which regulate NO production and vasodilation (Chachisvilis et al., 2006; Erdogmus et al., 2019). Interestingly, although GPR68 likely has a key role in regulating vasodilation, S1PR1 shows higher expression in ECs (Vanlandewijck et al., 2018). GPCRs such as GPR68, B2R, S1PR1, and H1R are the main GPCR contributors to endothelial mechanosensing; however, more studies are needed to distinguish whether they function as apex mechanosensors or mechanoregulatory proteins.

Adhesion Molecules

Integrins

Integrins are heterodimer transmembrane proteins that link the extracellular matrix (ECM) to the cell actomyosin cytoskeleton (thus integrating the cell cytoskeleton with the ECM) and are located at the basal surface of ECs (Hynes, 2004). Integrins are key cell adhesion mechanosignaling mediators that respond to shear stress through direct interaction with the ECM by undergoing significant conformational changes (integrin activation) (Driscoll et al., 2021). Following activation, integrins cluster with ECM fibrils, forming focal adhesions, and bind to cytoskeletal fibres (Kanchanawong et al., 2010; Schwartz, 2010). The Rho family of small GTPases regulate the assembly of focal adhesion complexes and cytoskeletal organization downstream of integrin activation (Shyy and Chien, 1997; Tzima, 2006; Tzima et al., 2001). The downstream biochemical activation of mechanosensory adhesion molecules results in the recruitment of multiprotein complexes called integrin adhesion complexes (IACs) (Byron et al., 2012). In response to shear stress, signalling through IACs is bidirectional (inside-out and outside-in) across the plasma membrane and regulates different EC processes, including morphology changes, migration, proliferation, activation and gene expression (Shyy and Chien, 2002). However, how shear stress directly mediates integrin activation is still largely unknown. Studies in cultured cells have proposed that integrin clustering and activation might occur via direct mechanical stretching of ECs or be mediated through remodelling of the ECM (Ross et al., 2013).

Integrins consist of 18 α and 8 β subunits and are present in cells as 24 unique heterodimers with unique ligand binding and intracellular signalling characteristics

(Chastney et al., 2025). Shear stress mediates integrin activation and phosphorylation of key kinases involved in regulation of cell adhesion, including tyrosine phosphorylation of focal adhesion kinase (FAK) and Src family protein kinases (c-Src and p130Cas), all of which are suggested to be involved in actin reorganization and further activation of downstream kinases, such as extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK) and NF-kB (Jiang et al., 2021; Kim et al., 2011; Mengistu et al., 2011; Orr et al., 2005). Integrin activation is therefore central in the EC response to shear stress. For example, acute onset of shear stress mediates αvβ3 integrin-mediated kinase phosphorylation (p-NFκB S536, p-FAK Y397) and expression of vascular cell adhesion molecule 1 (VCAM-1), and intercellular cell adhesion molecule 1 (ICAM-1)[98]. Furthermore, inhibition of αvβ3 integrin activation prevents early onset of atherosclerosis, suggesting that these responses play a proinflammatory and pathogenic role in atherosclerosis (Chen et al., 2015). By contrast, β1 integrin is involved in physiological EC alignment and actin remodelling in response to high unidirectional (laminar) shear stress (Macek Jilkova et al., 2014). Mechanistically, acute laminar shear stress induces transposition of β1 integrin to caveolae associated with activation of Src via Y416 phosphorylation, C-terminal Src kinase (Csk) induction and myosin light chain phosphorylation (Radel et al., 2007).

The type of integrin heterodimer that is activated is dictated by ECM composition and shear stress (Jalali et al., 2001; Orr et al., 2005). The major ECM proteins that bind to integrins are collagens and laminins, which are usually associated with normal physiological EC function within the vascular wall (Hynes, 2002). In contrast, fibronectin, fibrinogen and vitronectin, which have been shown to be deposited in response to acute shear stress or inflammation, bind to α5β1, αvβ3, αvβ5 and α8β1 integrin heterodimers and are associated with EC activation, inflammation and proliferation *in vitro* (Béguin et al., 2020; Feaver et al., 2010; Hynes, 2004; Saemisch et al., 2019; Thoumine et al., 1995). Interestingly, *in vitro* studies by Tzima et al. and others suggested that shear stress directly mediates an increase in binding of newly-deposited integrin to the ECM, which is subsequently responsible for EC adaptation to flow (Mott and Helmke, 2007; Tzima et al., 2001). Because matrix composition and stiffness vary between vascular beds, flow-mediated integrin activation might differ substantially between arteries, veins, capillaries and lymphatics. Moreover, a significant gap in understanding exists regarding how shear stress – particularly

disturbed low-amplitude shear stress – can promote integrin-mediated ECM deposition and how this influences EC activation and inflammation in atheroprone areas *in vivo*.

Plexin D1 mechanosensory complex

Plexin D1 (PLXND1) is a member of the semaphorin-binding cell guidance receptor family that regulates cellular patterning by modulating the cytoskeleton and focal adhesions (Aghajanian et al., 2014; Sakurai et al., 2010). Its canonical mode of activation is through binding of semaphorins (Rozbesky and Jones, 2020); however, recent work has revealed that PLXND1 also acts as a force sensor in ECs independent of its ligand-binding function (Mehta et al., 2020). PLXND1 couples with co-receptors vascular endothelial growth factor receptor 2 (VEGFR2) and neuropilin-1 (NRP1) to form a mechanosensory complex that acts upstream of the PECAM-1-based junctional complex and integrins (Mehta et al., 2020). Crucially, heterologous expression of PLXND1, NRP1 and VEGFR2 in Cos7 cells confers responsiveness to flow. Direct force application on PLXND1 activates several key fluid shear stress-induced signalling responses, including activation of VEGFR2, ERK1/2 and Akt, whereas loss of PLXND1 in ECs results in impaired shear stress responses (Mehta et al., 2020). Elegant structural and mutagenesis studies revealed that PLXND1 exhibits distinct molecular conformations that are key to distinct modes of activation induced via either its ligands or mechanical stresses (Mehta et al., 2020). The relevance of the mechanosensing role of PLXND1 in disease pathogenesis has been investigated in a preclinical mouse model of atherosclerosis: in this study, deficiency of endothelial *Plxnd1* was protective against the development of atherosclerotic lesions in areas of disturbed flow via inhibition of endothelial pro-inflammatory activation (Mehta et al., 2020).

PECAM-1 junctional mechanosensory complex

PECAM-1, an adhesion molecule in the immunoglobulin superfamily, plays a key role in EC function (Newman and Newman, 2003). The PECAM-1 extracellular domain enables homophilic binding and its intracellular domain contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Phosphorylation of PECAM-1, along with Ca^{2+} influx and K^{+} channel activation, is an early shear stress response (Givens and Tzima, 2016): upon shear stress, ITIM tyrosines (Y663 and Y686) are rapidly phosphorylated, independent of Ca^{2+} influx. PECAM-1 forms a junctional mechanosensory complex

with VE-cadherin and VEGFR2 to regulate EC alignment and downstream signalling pathways (Newman and Newman, 2003). Ectopic expression of PECAM-1, VE-cadherin, and VEGFR2 in heterologous cells that are typically unresponsive to flow showed that these factors are sufficient to reconstitute responsiveness to shear stress (Tzima et al., 2005). Additionally, PECAM-1 influences EC polarity via Rac GTPase activation and also modulates eNOS localization, impacting NO production (Bagi et al., 2005; Chen et al., 2010) . Like PIEZO1, PECAM-1 appears to be involved in EC responses to both physiological shear stress and pathology-associated low shear stress: hypercholesterolemic mice with EC-specific deletion of *Pecam-1* consistently exhibited increased atherosclerosis at sites of physiological shear stress and decreased atherosclerosis at sites of low shear stress (Goel et al., 2008; Harry et al., 2008; Stevens et al., 2008).

Recent studies have begun to reveal that adhesion molecules and PIEZO1 can form protein complexes (Chuntharpursat-Bon et al., 2023b; Koster et al.; Wang et al., 2022a). PECAM-1, VE-cadherin and PIEZO1 were found to be in a complex located at cell-cell junctions. At this junctional compartment, PIEZO1 was shown to regulate the movement of Ca^{2+} from the extracellular interstitial space between adjacent cells to the intracellular face of the junction. Formation of VE-cadherin junctions was dependent on this extracellular Ca^{2+} pool, and cortical actin remodelling on the intracellular face of the junction was also Ca^{2+}-dependent. Knockdown of PIEZO1 produced tighter VE-cadherin junctions, indicative of high Ca^{2+} levels in the extracellular compartment, and stabilised cortical actin due to reduced Ca^{2+} entry on the intracellular face. PECAM-1 was also shown to suppress PIEZO1 activity (Chuntharpursat-Bon et al., 2023a). Furthermore, PIEZO1 forms complexes with cell adhesion molecules 1 and 4 (CADM1 and CADM4, respectively) (Koster et al., 2024) and E-cadherin (Wang et al., 2022b).

HEG1

The heart development protein with EGF-like domains 1 (HEG1) is a single-pass transmembrane glycoprotein enriched in ECs. During development, HEG1 plays a key role in cardiac development and EC integrity. Global HEG1 knockout in zebrafish and mice causes embryonic death due to cardiac enlargement, ventricular thinning and "leaky" vessels (Kleaveland et al., 2009; Mably et al., 2003; Stainier et al., 1996).

In adult aortic ECs, HEG1 maintains its homeostatic function under uniform flow conditions by inducing expression of Krüppel-like factors 2 and 4 (KLF2 and KLF4, respectively), which provides atheroprotection (Tamargo et al., 2024). HEG1 enhances induction of KLF2 and KLF4 by activating the MEKK3-MEK5-ERK5-MEF2c pathway; however, the mechanism by which HEG1 activates MEKK3 remains unclear, although it is known to involve cerebral cavernous malformation 1 (CCM1, also known as KRIT1) and CCM2 (Iruela-Arispe, 2024). Upon exposure to uniform flow, HEG1 quickly (within minutes) translocates from the cytoplasm to cell-cell junctions positioned downstream from flow; in cultured human aortic endothelial cells (HAECs), it is also released from the cell and can be detected in the culture media. The significance of HEG1 translocation to the cell junction and its release into the media requires further research. However, siRNA-mediated HEG1 knockdown in HAECs *in vitro* significantly reduces the effects of uniform flow on the endothelial permeability barrier, including attenuating protective, anti-inflammatory and anti-migratory responses. Additionally, tamoxifen-inducible, endothelial-targeted HEG1 knockout ($HEG1^{iECKO}$) in mice leads to an increased permeability of low density lipoprotein (LDL) in regions exposed to disturbed flow, consistent with *in vitro* findings (Tamargo et al., 2024).

Most importantly, $HEG1^{iECKO}$ mice show an exacerbation of atherosclerotic plaque development, including thin-capped fibroatheroma (advanced atherosclerotic lesions with necrotic material, cholesterol crystals and inflammatory cells that are at increased risk of rupture) and intraplaque haemorrhage in the acute partial carotid ligation model (Tamargo et al., 2024). Further, loss of endothelial HEG1 expression was observed in human coronary arteries with advanced atherosclerotic plaques, demonstrating the clinical significance of HEG1 in human atherosclerosis (Tamargo et al., 2024). However, the functional importance, regulatory mechanisms and signalling mechanisms of HEG1 in vascular biology and diseases such as atherosclerosis under various flow conditions remain unclear, opening the door for additional studies.

EphB4

The ephrin receptor (Eph) family is a group of mechanoregulatory receptor tyrosine kinases (RTKs), comprising the EphA and EphB subfamilies. These receptors interact with cell surface proteins called ephrins and are essential in controlling cell adhesion, migration, tissue patterning and development, including axon guidance but also

angiogenesis (Mosch et al., 2010). Ligand-dependent ephrin type-B receptor 4 (EphB4) signalling is required to maintain the structural integrity of coronary capillaries, contributing to the structural integrity of ECs in response to mechanical forces by regulating focal adhesions (Luxán et al., 2019). Loss-of-function mutations of the *EPHB4* gene are associated with anomalies in capillaries and cerebral vessels (Amyere et al., 2017). Most notably, loss of EphB4 or Rasa1 (a Ras-GTPase activating protein) leads to vein of Galen malformations (Revencu et al., 2008), the most common cerebrovascular malformation in neonates. In a zebrafish model reproducing these mutations, the malformations were shown to be a consequence of defective fusion of progenitor vessels (Martin-Valiente et al., 2025). This mechanism was dependent on flow, indicating that flow also contributes to vasculogenesis by promoting progenitor vessel fusion. Loss-of-function of RASA1 in ECs induced an imbalance in flow-mediated activation of ERK and Akt but not of VEGF pathways. Restoration of these flow-mediated signalling cascades with drugs that activated PI3K or inhibited MEK1 reversed malformations in the zebrafish model of vein of Galen malformations (Martin-Valiente et al., 2025), demonstrating that pharmacologically targeting mechanotransduction of flow stimuli could be a promising therapeutic approach for this pediatric disease (Hale and Kahle, 2025). More research is needed to elucidate the link between flow and EphB4, as well as its interaction with RASA1 and the resulting signalling cascade.

TGF-beta receptors

The transforming growth factor (TGF)-beta family is a class of serine/threonine kinase receptors that form heteromeric complexes (Type I, Type II, and Type III receptors). These receptors bind TGF-beta and bone morphogenic protein (BMP) ligands, activating the Smad family of transcription factors (O'Connor and Gomez, 2014; Shi and Massagué, 2003). The activation of TGF-beta receptors has been demonstrated to be directly modulated by flow: in response to laminar physiological flow, Alk1 (a Type I receptor) and endoglin (a Type III receptor) interact, increasing their affinity for BMP9 (Baeyens et al., 2016). Disruption of Alk1 or endoglin leads to the formation of abnormal arteriovenous shunts in areas of high flow during development, hinting at a role for these receptors in flow-mediated vasculogenesis (Baeyens et al., 2016). It has been proposed that Alk1 and endoglin control both the flow-mediated repression of

endothelial proliferation and cell migration during angiogenesis (Baeyens et al., 2016; Jin et al., 2017; Sugden et al., 2017).

Alk1 and endoglin activate Smad1, Smad5 and Smad9 signalling, which has been demonstrated to be maximally activated at physiological values of shear stress (approximating the shear stress set point), thereby promoting vascular homeostasis (Baeyens et al., 2015). Elevation of shear stress, which stimulates vessel enlargement to maintain the shear stress set point, increases KLF2 expression, which mediates the transcription of BMP-binding endothelial regulator (BMPER), an inhibitor of Smad1 and Smad5 signalling (Deng et al., 2024). Other studies have also shown that Alk1 and endoglin repress vessel enlargement in response to increased flow through the regulation of endothelial cell size (Diwan et al., 2024). Baeyens et al. found that shear stress synergises with the BMP9–Alk1–endoglin signalling axis, whereby exposure to shear stress markedly enhances cellular responsiveness to minimal levels of BMP9 (Baeyens et al., 2016). A recent preprint proposes that flow-induced activation of Smad1 and Smad5 signalling is mediated through the activation of the GPCR latrophilin-2, which forms a complex with SH3 and multiple ankyrin repeat domains 3 (Shank3) and endoglin in the presence of physiological flow (Tanaka et al., 2025).

Reduction of flow leads to reduced shear stress and inward remodelling of blood vessels, indicating that specific signal transduction pathways are induced in response to low shear stress (Baeyens and Schwartz, 2016). Smad2 and Smad3 are maximally translocated to the EC nucleus in response to low shear stress, whereas their translocation is repressed at physiological values of shear stress (Deng et al., 2021). In other non-EC cell types, Smad2 and Smad3 signalling is involved in numerous repair processes, such as, for example, fibrosis, in which TGFBR1 (also known as Alk5) is the classical Type I TGF-beta receptor involved (Kim et al., 2018). Interestingly, the activation of Smad2 and Smad3 by low shear stress is associated with the flow-induced interaction of Alk5 with NRP1 (a Type III receptor) and BMP9 as the ligand. Smad2 and Smad3 signalling is repressed at physiological values of shear stress through inhibitory phosphorylation of their linker regions by the MEKK3-KLF2 pathway (Deng et al., 2021). Endothelial-specific deletion of MEKK3 leads to spontaneous arterial inward remodelling and hypertension, which is rescued in a MEKK3 and Alk5 double knock-out mouse model (Deng et al., 2021). All these observations point to an essential

contribution of TGF-beta receptors in the control of vascular homeostasis and remodelling and provide a first mechanistic insight into the shear stress set point mechanism.

Primary Cilia

Primary cilia are solitary, immotile, antenna-like sensors of extracellular mechanical and chemical stimuli, found on most quiescent or differentiated mammalian cell types (Singla and Reiter, 2006). Measuring several microns in length, they consist of a ciliary membrane, an axoneme containing nine doublet microtubules, and a basal body (a modified mother centriole). Primary cilia have been indicated to function as flow sensors in the kidney, bone, bile duct and the cardiovascular system (Praetorius, 2015).

By analysing flow responses of ciliated compared to non-ciliated chicken embryonic ECs, Hierck et al. demonstrated that primary cilia sensitize ECs to fluid shear stress (Hierck et al., 2008). Similarly, cilia were shown to mediate the response of cultured mouse aortic ECs to the onset of flow (characterized by an increase in cytosolic Ca^{2+} followed by release of NO) via ciliary proteins polycystin-1 (PKD1) and polycystin-2 (PKD2) (AbouAlaiwi et al., 2009; Nauli et al., 2008). In zebrafish embryos, EC cilia detect low shear forces and regulate angiogenesis via PKD2 and Ca^{2+} signaling (Goetz et al., 2014). It has been postulated that PKD1 and PKD2 form a mechanosensory channel on the cilium: PKD1, a transmembrane glycoprotein, might serve as a direct sensor of force, with PKD2, a TRP channel, transducing the force through Ca^{2+} signaling (Nigro and Boletta, 2021). Vion et al. described another ciliary flow-sensing mechanism in the developing mouse retina, where primary cilia sensitize ECs to BMP9 under low flow conditions to regulate vascular patterning (Vion et al., 2018).

The presence of primary cilia on ECs is correlated with shear stress in large arteries. In the chicken embryonic heart, primary cilia are present in regions with low shear stress, and primary cilia distribution is inversely correlated with expression of the high shear stress marker KLF2 (Van der Heiden et al., 2006). Moreover, Van der Heiden et al. detected primary cilia at atherosclerosis-prone sites characterised by disturbed flow, including the inner curvature of the aortic arch, branch points of the aorta, the sinus of the internal carotid artery and aortic valve leaflets (Van der Heiden et al., 2008). To

confirm the correlation between disturbed flow and the presence of primary cilia, the authors induced disturbed flow in mouse carotid arteries using a constrictive cast (Cheng et al., 2006). Intriguingly, the number of EC primary cilia increased in regions of low and low/multidirectional shear stress within two days of cast placement (Van der Heiden et al., 2008). Loss of EC cilia leads to misalignment of ECs (Vion et al., 2018), decreased eNOS activity and increased pro-inflammatory gene expression, and predisposes mice to development of atherosclerosis (Dinsmore and Reiter, 2016). Loss of EC cilia also increased atherosclerosis in hypercholesterolemic mice, indicating that primary cilia protect against atherosclerosis (Dinsmore and Reiter, 2016). Further studies are warranted to elucidate the mechanisms by which EC cilia sense and transduce shear stress and protect against cardiovascular disease.

Glycocalyx

The extracellular endothelial surface glycocalyx is a crucial mechanotransducer, enabling shear stress to regulate EC motility and proliferation (Yao et al., 2007) and other activities, such as permeability and inflammation, that either protect against or contribute to disease (Cancel et al., 2016; Ebong et al., 2011; Tarbell and Cancel, 2016). The ability of the glycocalyx to mediate mechanotransduction stems from its complex structure: a hydrated, negatively charged network of sugar chains that encapsulate ECs, with prominence on the apical surface of the endothelium (Cancel et al., 2016; Ebong et al., 2011; Tarbell and Cancel, 2016). These sugar chains include heparan sulphate, hyaluronic acid and chondroitin sulphate (Banerjee et al., 2021; Harding et al., 2018; Mitra et al., 2022; Tarbell and Pahakis, 2006). The glycocalyx also contains glycoproteins, glycolipids and proteoglycans, such as membrane-bound glypican-1 and transmembrane syndecan-1, which anchor the sugar chains to the cell membrane and cytoskeleton, respectively (Banerjee et al., 2021; Harding et al., 2018; Mitra et al., 2022; Tarbell and Pahakis, 2006).

The glycocalyx interacts extensively with mechanotransduction proteins. For example, it exhibits important interactions with caveolae rafts in the cell membrane. Disturbed fluid shear stress suppresses expression of glycocalyx components, particularly heparan sulphate; compared to uniform shear stress, it also leads to decreased caveolin-1 expression, reduced caveolin-1 localization at the EC membrane and impaired caveolin-1 colocalization with the activated form of eNOS, which is regulated

by caveolae residency (Harding et al., 2018). These alterations contribute to pro-atherosclerotic endothelial dysfunction under disturbed shear stress conditions. Similarly, obesity-induced endothelial dysfunction has been linked to glycocalyx degradation, which impairs the flow-sensitivity of Kir2.1 channels, thereby compromising EC responses to fluid shear stress and the ability to induce vasodilation and regulate blood pressure (Fancher et al., 2020).

Additional studies further highlight the role of the glycocalyx in facilitating shear-induced endothelial signalling in partnership with other key players. For instance, heparan sulphate is necessary for the formation of the mechanosensitive PECAM-1 and Gαq/11 complex, which governs shear stress-induced Akt activation (dela Paz et al., 2014). Disrupting heparan sulphate impairs PECAM-1 interactions with Gαq/11, weakening downstream mechanotransduction (dela Paz et al., 2014). Likewise, glypican-1, a core glycocalyx protein responsible for anchoring heparan sulphate, responds to fluid shear stress by phosphorylating PECAM-1, leading to eNOS activation and NO production (Bartosch et al., 2021). More recent evidence suggests that ENaC (discussed above) is physically linked to the glycocalyx and collaborates with it to regulate NO production and vascular tone in response to shear stress (Cosgun et al., 2022; Fronius, 2022).

These findings highlight the essential role of the glycocalyx in mechanotransduction, particularly in NO regulation, as well as a broader role in various other endothelial functions not emphasised here. As glycocalyx degradation disrupts these functions and contributes to endothelial and vascular dysfunction, specifically in atherosclerosis, further research is needed to fully understand its mechanistic role. Given a growing interest in glycocalyx-targeted therapies, such as strategies to preserve surface expression of heparan sulphate (Mitra et al., 2024), there is potential for novel interventions to slow atherosclerosis progression by restoring glycocalyx-mediated endothelial function.

Membrane Fluidity

Flow also elicits mechanosignalling by modifying the fluidity of the plasma membrane. This process involves stearoyl-CoA desaturase 1 (SCD1), which converts saturated fatty acids into monounsaturated fatty acids (MUFAs) such as oleic and palmitoleic

acids, and is regulated by peroxisome proliferator-activated receptor (PPAR)-gamma (Lu et al., 2020; Qin et al., 2007) (Fig. 3). Endothelial SCD1 is activated by uniform, unidirectional shear stress (Aengevaeren et al., 2020), promoting MUFA production (Cavallero et al.; Qin et al., 2007) to maintain membrane fluidity and also promoting anti-inflammatory pathways. By contrast, disturbed flow conditions downregulate SCD1-catalysed fatty acid metabolites (oleic and palmitoleic acids), attenuating vascular protective effects (Cavallero et al.). Notably, exercise, a physiological driver of shear stress, activates SCD1 expression in the ECs of the aortic arch and descending aorta, where ECs are exposed to oscillatory and pulsatile shear stress, respectively (Cavallero et al.) (Fig. 3). Furthermore, exercise-augmented shear stress activates endothelial SCD1 to mitigate inflammatory biomarkers (e.g., ICAM-1 and the cytokine MCP-1) (Cavallero et al.). The NIH Molecular Transducers of Physical Activity Consortium (MoTrPAC) has reported the exercise-activated multi-omics response to endurance exercise training. This report highlights the flow-regulated SCD1-MUFA pathway as potential therapeutic targets to treat vascular dysfunction for individuals who are physically limited to engaging in exercise (MoTrPAC Study Group et al., 2024).

Conclusion

ECs are exquisitely sensitive to mechanical cues associated with blood flow. In this Review, we focus on one such cue, shear stress, which ECs sense and decode in terms of magnitude, direction and temporal dynamics. We have highlighted progress in the vascular mechanobiology field in identifying multiple mechanoreceptors involved in sensing of shear stress by ECs. However, despite this progress, the mechanisms that allow ECs to distinguish between shear stress conditions of differing magnitude, direction and timing are largely unknown.

Some evidence indicates that specific mechanical stimuli elicit specific responses by activating distinct sets of mechanoreceptors. In support of this concept, β1 integrin can be activated by unidirectional force but is unresponsive to bidirectional force. Moreover, the transmembrane heparan sulphate proteoglycan syndecan-4 is required for EC alignment under shear stress but is dispensable for other mechanoresponses, indicating a role in sensing of flow direction (Baeyens et al., 2014). However, there is also evidence that a range of mechanical conditions can activate some

mechanoreceptors but elicit specific downstream responses according to the nature of the force applied. For example, PECAM-1 and PIEZO1 (Albarrán-Juárez et al., 2018; Zhu et al., 2024) can sense both unidirectional and disturbed flow, leading to the transmission of protective and inflammatory signals accordingly. Therefore, it appears that ECs mount highly specific responses to mechanical perturbation through a complex process involving a subset of mechanoreceptors that integrate to elicit downstream signalling that is specifically tuned to the mechanical environment. Further research is needed to generate an integrated model of shear stress sensing to understand how mechanoreceptors functionally interact to maintain vascular homeostasis under different flow conditions.

An additional key question in the vascular mechanobiology field relates to variations in mechanical sensitivity according to anatomical variation and disease. This has often been framed according to the shear stress set point theory, which suggests that different vessels have different shear stress 'comfort zones' (Baeyens and Schwartz, 2016; Baeyens et al., 2015). For example, arteries undergo outward remodelling in response to enhanced blood flow and inward remodelling in response to reduced blood flow, resulting in the restoration of homeostatic shear stress (which in humans is approximately 1Pa). This is supported by *in vitro* studies showing that arterial ECs are quiescent at 1Pa shear stress but become activated by low or supraphysiological shear stress. By contrast, venous and lymphatic EC are quiescent at lower shear stress magnitudes, suggesting that they have a different set point (Deng et al., 2021). Despite multiple observations supporting the set point theory, the mechanistic basis at the level of mechanoreceptor activation is unclear and requires further research.

Similarly, shear stress responses appear to differ between pathogenic states. This is evidenced in the field of atherosclerosis where diseased vessels undergo aberrant remodelling. This might occur in part through downregulation of mechanoreceptors in atherosclerosis, as shown for the protease kallikrein-10 (KLK10) (Williams et al. 2022), which is expressed at regions of physiological shear stress in healthy arteries but is absent from regions of high shear stress at plaques. Genetic disruption of some genes also leads to vascular anomalies such as malformed vascular networks. For example, the formation of direct fast-flow shunts between arteries and veins (Hereditary Hemorrhagic Telangiectasia) has been observed to be associated with genetic and/or

somatic mutations of components of the BMP9 and BMP10 signaling pathway (Alk1, endoglin, BMP9 or Smad4) (DeBose-Scarlett and Marchuk, 2025). Other vascular anomalies, such as capillary malformation-arteriovenous malformation syndrome and vein of Galen malformations, associated with germline mutations of *EPHB4* (Amyere et al., 2017) or *RASA1* (Revencu et al., 2008; Revencu et al., 2013), exhibit malformed capillary structures and, in some patients, an abnormal formation of the vein of Galen resulting from defective flow-mediated fusion of progenitor vessels (Martin-Valiente et al., 2025). These loss-of-function mutations demonstrate the importance of these genes in the formation of a correctly structured vascular network and the role of flow sensing in these processes. Moreover, further work is needed to mechanistically link the myriad mechanosensitive transcripts and proteins to upstream mechanosensors.

Overall, further work is needed to understand how disease risk factors integrate with mechanoreceptor expression and function, particularly in relation to the nature of the vascular beds, the cell phenotype, flow parameters and the underlying genetic background.

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FIGURE LEGENDS

Fig. 1: Characterisation of shear stress in the vasculature.

Variation in blood vessel size and shape generates differential flow patterns, such as disturbed flow in areas of bifurcation and curvature and uniform flow in regions of the vessel with uniform geometry. Left: Uniform flow generates shear stress that is unidirectional with relatively high magnitude. Right: Disturbed flow generates a more complex shear stress field, characterised by lower magnitude (low shear stress), oscillations in direction (oscillatory shear index, OSI) and flow that is perpendicular to the vessel wall (transverse shear stress). Oscillatory shear stress resulting from disturbed flow is atherogenic (e.g. it promotes the development of atherosclerotic disease).

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Fig. 2: Endothelial mechanosensors.

An overview of mechanosensors and mechano-regulatory proteins in endothelial cells. Several mechanosensitive ion channels contribute to sensing shear stress in the endothelium. Apex mechanosensors, such as PIEZO1, can detect a variety of forces in different subcellular locations. Upon force activation, PIEZO1 undergoes a conformational change that causes the plasma membrane to flatten and the ion pore to open (top right). PIEZO1 is inherently mechanosensitive but can also interact with other mechano-regulatory proteins, such as the adhesion molecules PECAM1 and VE-cadherin (CDH5), at cell-cell junctions (bottom left). Other flow-sensitive mechano-regulatory factors in endothelial cells include TGF-β receptors, G-protein coupled receptors (GPCRs), primary cilia and the glycocalyx. In addition, endothelial cell plasma membrane fluidity responds to shear stress, activating downstream metabolic pathways.

Fig. 3. Exercise enhances endothelial cell metabolic function via pulsatile shear stress.

The development of disturbed or atherogenic flow, which generates oscillatory shear stress (OSS), occurs at the lesser curvature of the aortic arch. In contrast, atheroprotective pulsatile shear stress (PSS) manifests in the descending aorta. Exercise augments PSS, which diminishes the oscillatory shear index (OSI) in the lesser curvature. This change in the OSI is transduced via EC mechanosensors like

PIEZO1 and activates SCD1 through a PPARγ-dependent mechanism, facilitating the catalysis of lipid metabolites. ECs metabolise free fatty acids (FFA) or albumin-bound fatty acids (albumin-FA). SCD1, localised within the mitochondria-associated membrane (MAM), a contact site between the endoplasmic reticulum (ER) and mitochondria, governs the conversion of saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA), which maintain membrane fluidity. The SCD1-mediated production of the MUFA oleic acidattenuates NFκB-driven vascular inflammation and ER stress. Abbreviations: sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), mitochondrial pyruvate carrier (MPC), tricarboxylic acid cycle (TCA), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). LSS, low shear stress.

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