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REVIEW

Biophysical and Biochemical Roles of Shear Stress on Endothelium: A Revisit and New Insights

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ABSTRACT: Hemodynamic shear stress, the frictional force exerted by blood flow on the endothelium, mediates vascular homeostasis. This review examines the biophysical nature and biochemical effects of shear stress on endothelial cells, with a particular focus on its impact on cardiovascular pathophysiology. Atherosclerosis develops preferentially at arterial branches and curvatures, where disturbed flow patterns are most prevalent. The review also highlights the range of shear stress across diverse human arteries and its temporal variations, including aging-related alterations. This review presents a summary of the critical mechanosensors and flow-sensitive effectors that respond to shear stress, along with the downstream cellular events that they regulate. The review evaluates experimental models for studying shear stress in vitro and in vivo, as well as their potential limitations. The review discusses strategies targeting shear stress, including pharmacological approaches, physiological means, surgical interventions, and gene therapies. Furthermore, the review addresses emerging perspectives in hemodynamic research, including single-cell sequencing, spatial omics, metabolomics, and multiomics technologies. By integrating the biophysical and biochemical aspects of shear stress, this review offers insights into the complex interplay between hemodynamics and endothelial homeostasis at the preclinical and clinical levels.

Key Words: atherosclerosis ■ endothelial cells ■ exercise ■ hemodynamics ■ multiomics

The frictional force exerted by blood flow on the surface of endothelial cells (ECs) is known as hemodynamic shear stress. This force plays a pivotal role in maintaining endothelial homeostasis by influencing a range of cellular events, including proliferation, cell death, inflammatory responses, activation, and transdifferentiation.[1] In the late 15th century, Leonardo da Vinci first documented the potential significance of hemodynamics in the cardiovascular system, by suggesting that the heart's blood flow might resemble the principles observed in the water flow around obstacles in a riverbed.[2] Subsequently, in the mid-19th century, a hypothesis was proposed that atherosclerosis is caused by mechanical irritation on the arterial wall. Until the mid-to-late 20th century, it was thought that the bloodstream exerted a shearing force, termed shear stress, on the arterial wall, which altered endothelial morphology and alignment.[3] In the 21st century, however, hemodynamic shear stress has been identified as a crucial contributor to endothelial homeostasis and the pathophysiology of atherosclerotic cardiovascular diseases, including ischemic stroke, myocardial infarction, and peripheral artery disease. The advances in biomedical and omics technologies have greatly facilitated the identification of the functional, structural, transcriptomic, epigenomic, and metabolic roles of shear stress on ECs (Figure 1).

Hemodynamic shear stress can be classified into 2 main categories: steady laminar shear stress (LSS) and oscillatory shear stress (OSS). LSS is defined by a unidirectional flow pattern, which is typically observed in straight arterial regions, with a value of ≈15 dyn/cm^2. In particular, LSS has been demonstrated to suppress endothelial activation and preserve endothelial morphology, eliciting anti-inflammatory and antiatherogenic effects.[4] In contrast, OSS is defined by a bidirectional or multidirectional flow pattern, occurring in sites with disturbed flow, namely curvatures, stenosis, and bifurcations (≈±4 dyn/

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Nonstandard Abbreviations and Acronyms

3D	3 dimensional
AKT	protein kinase B
AVF	arteriovenous fistula
EC	endothelial cell
ECM	extracellular matrix
EndoMT	endothelial-to-mesenchymal transition
eNOS	endothelial NO synthase
ERK1/2	extracellular signal-regulated kinase 1/2
GPCR	G-protein–coupled receptor
HIF1α	hypoxia-inducible factor 1α
KLF	Krüppel-like factor
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
LSEC	liver sinusoidal endothelial cell
LSS	steady laminar shear stress
miRNA	microRNA
MMP	matrix metalloproteinase
NEAT1	nuclear paraspeckle assembly transcript 1
NF-κB	nuclear factor-κB
OSS	oscillatory shear stress
P2X4	P2X purinoreceptor 4
PAH	pulmonary arterial hypertension
PCL	partial carotid ligation
PCSK9	proprotein convertase subtilisin/kexin type 9
PEC	pulmonary endothelial cell
PECAM1	platelet endothelial cell adhesion molecule-1
PI3K	phosphoinositide 3-kinase
PIEZO1	piezo type mechanosensitive ion channel component 1
RCA	right carotid artery
scRNA-seq	single-cell RNA sequencing
SOX	SRY-box transcription factor
Src	tyrosine-protein kinase
TAZ	transcriptional coactivator with PDZ-binding motif
TF	transcription factor
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
WSS	wall shear stress
YAP	yes-associated protein

cm^2). The current understanding is that OSS exacerbates endothelial dysfunction, activation, and morphological changes, thereby accelerating the initiation and progression of atherosclerosis.[4] This review discusses the current understanding and future perspectives on the biochemical and biophysical roles of shear stress on the endothelium.

BIOPHYSICAL NATURE OF HEMODYNAMIC FLOW

Atherosclerosis, referring to the formation of fibrofatty plaques within the subendothelial space of the arterial wall, is a multifactorial disease driven by dyslipidemia and chronic inflammation, with the participation of hemodynamic flow. It is of particular importance to note that atherosclerotic plaques preferentially form at regions subjected to flow disturbance.[5] The bloodstream exerts multiple forms of mechanical stimuli to ECs, including tensile strain, compressive pressure, and shear stress (Figure 2A).

Blood pressure varies considerably within the human vascular system, with values ranging from ≈1.3 kPa (10 mm Hg) in the veins to ≈16 kPa (120 mm Hg) in the aorta during the contraction phase of the heart (systole). Blood pressure can reach as high as 27 kPa (200 mm Hg) in cases of severe hypertension. Blood pressure exerts a radial tensile force, whereby the difference in transmural pressure dilating blood vessels cyclically creates an internal circumferential (hoop) stress in the vascular wall.[6] In general, cardiac cycle–associated changes in circumferential strains due to diameter fluctuations range from 0% to 15%.[7] The circumferential stress plays a regulatory role in endothelial activation, alignment, and elongation.[7,8]Additionally, blood pressure also exerts a normal force on the apical surface of ECs.[7] This compressive force can alter morphology, cytoskeletal structure, angiogenesis, endothelial-to-mesenchymal transition (EndoMT), and vascular function-related signaling in ECs, as shown by in vitro experiments applying hydrostatic pressures on ECs.[7,9]

Hemodynamic shear stress refers to the tangential frictional force exerted by blood flow on a unit area of ECs (units: dyn/cm^2 and N/m^2). The presence of plasma and erythrocytes in THE blood confers a non-Newtonian property of blood and collectively contributes to the composite shear stress on ECs.[10] Differential time-averaged wall shear stress (WSS) is observed in aortas (≈10 dyn/cm^2), small arterioles (≈50 dyn/cm^2), venules (≈20 dyn/cm^2), and vena cava (≈1 dyn/cm^2).[7,11] It should bear in mind that shear stress magnitudes are not uniform over ECs of the same vessel, meaning that there is a shear stress gradient. Adjacent ECs are subjected to markedly distinct profiles of shear stress. Prolonged exposure to steady flow flattens EC surface, significantly reducing the peak shear stresses and shear stress gradients (≈40%), compared with ECs under static condition.[12] Furthermore, the presence of different geometric characteristics, such as branching and curvature, can cause fluctuations in shear stress magnitudes along the same vessel. In addition to influencing endothelial alignment

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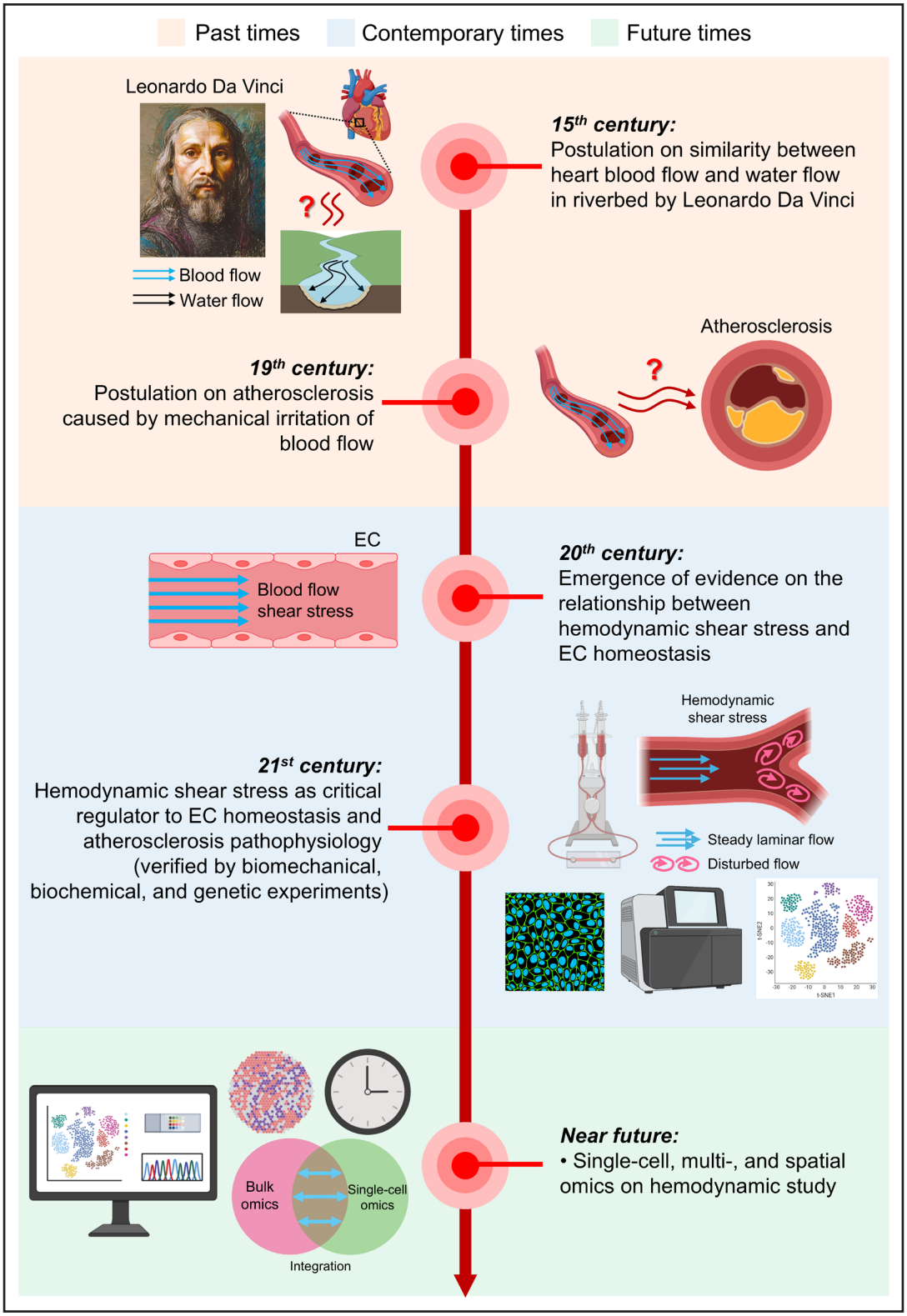

Figure 1. A brief history of hemodynamic study.
A review of the past, contemporary, and future contexts of hemodynamic studies on endothelial cells (ECs). Leonardo Da Vinci's portrait was created by Leonardo AI. Processing software: Biorender.

and morphology, shear stress regulates a number of EC functions, including the production of NO, angiogenesis, cell fate determination, and vascular remodeling.[13]

Hemodynamic flow is the movement of blood through the circulatory system. Different patterns (Figure 2B), dynamics (Figure 2C), and directions (Figure 2D) of

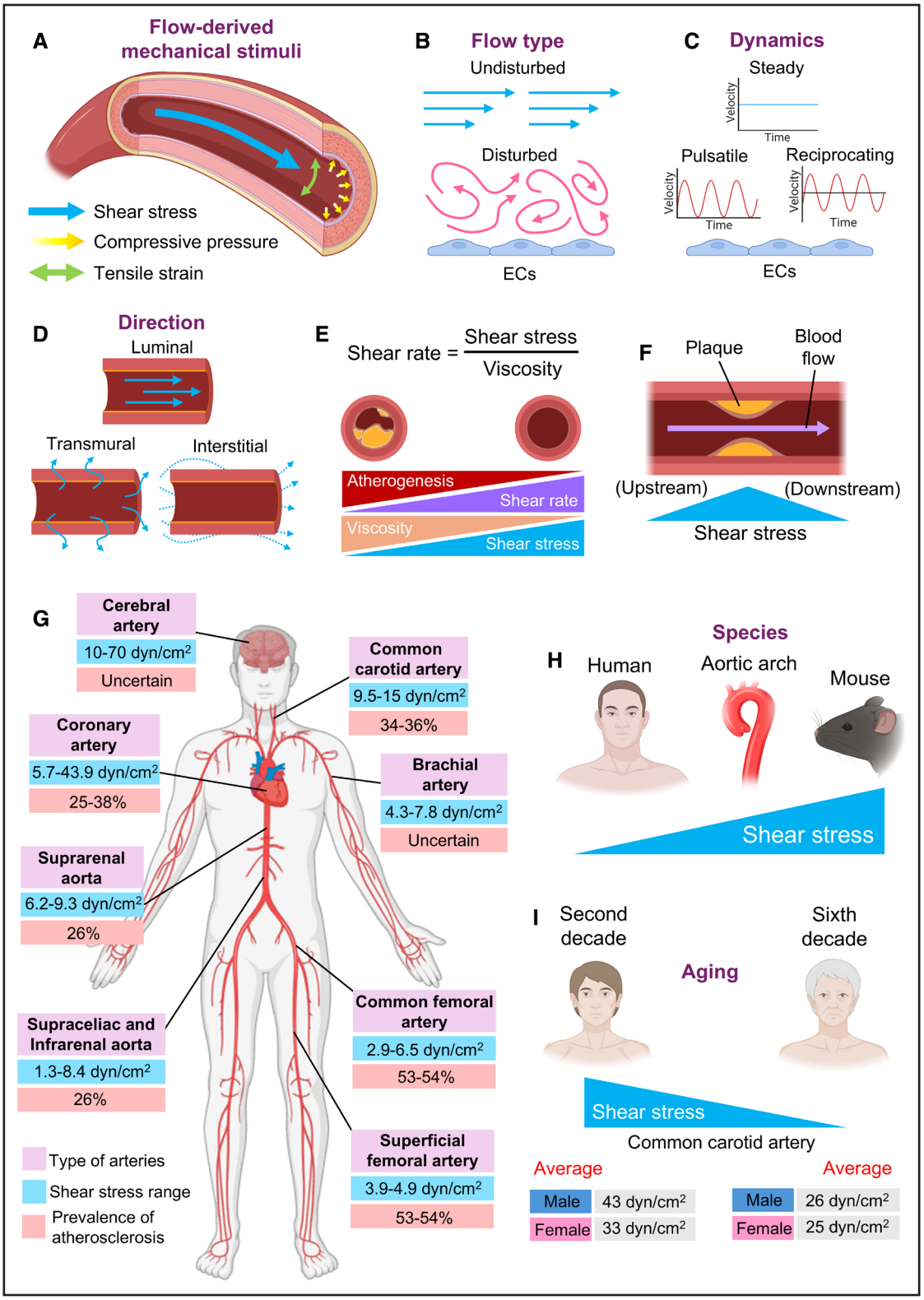

Figure 2. Biophysical features of hemodynamic flow and shear stress.
A, Exertion of various forms of mechanical stress on endothelial cells (ECs) by hemodynamic flow, including shear stress, blood pressure, and tensile strain. **B**, Types. **C**, Dynamics. **D**, Directions of hemodynamic flow experienced by ECs. **E**, The relationship between hemodynamic parameters (ie, shear rate, shear stress, and viscosity), and corresponding atherosclerosis risk. **F**, Variation of shear stress in the upstream, midstream, and downstream portions of atherosclerotic plaque. **G**, Shear stress ranges and prevalence of atherosclerosis in different types of human arteries. **H**, Variation of shear stress magnitudes between species, such as the human and mouse. **I**, A decline in shear stress intensities has been observed in the common carotid arteries of both male and female individuals during the aging process. Processing software: Biorender.

hemodynamic flow correspond to different magnitudes and directions of shear stress. In general, blood flow can be categorized into 2 main types, undisturbed or disturbed (Figure 2B). Undisturbed flows are primarily associated with steady laminar and uniform patterns, whereas disturbed flows encompass both laminar and turbulent flows, the latter of which are characterized by spatial shear stress gradients and secondary flow patterns.[7,14] In regions of the vasculature where the WSS is high, undisturbed flow tends to occur in a relatively straight configuration, with the flow streamlines remaining largely parallel to the vascular walls.[15]

Conversely, in regions of the vasculature where the WSS is low, and where there is a high degree of branching, curvature, bifurcation, and stenosis, the flow becomes significantly disturbed, resulting in irregular profiles such as flow separation, recirculation, and reattachment.[15,16] It is important to note that undisturbed flow serves to protect endothelial barrier and enhance endothelial resilience, while disturbed flow has the opposite effect, disrupting the endothelial barrier and promoting endothelial dysfunction, which in turn elicits proinflammatory and proatherogenic effects. As a result, vascular areas of flow disturbance are often vulnerable to the onset of vascular abnormalities and disorders, including inflammation, atherosclerosis, aortic valve calcification, and thrombosis.[16]

Arising from the rhythmic contraction of the heart, the blood flow in large vessels is pulsatile. Meanwhile, this pulsatility observed in the macrovasculature becomes damped in the microvasculature, resulting in a quasi-steady state of blood flow.[17] In other words, the dynamics of shear stress are highly dependent on the vascular regions. In undisturbed flow-exposed medium and large vessels, blood flow is pulsatile and nonreversal. In contrast, in disturbed flow regions, blood flow is generally reversing pulsatile and reciprocating. The diverse forms of flow patterns, including steady, reversing pulsatile, nonreversing pulsatile, and reciprocating flows (Figure 2C),[18] exert differential effects on the genotypes-phenotypes of ECs. Following prolonged exposure to unidirectional steady and nonreversing pulsatile flow, ECs undergo elongation and alignment along the flow direction, which impedes endothelial activation.[19] Conversely, exposure to reversing pulsatile or reciprocating flow disrupts this elongation and orientation, thereby facilitating a proinflammatory and proatherogenic phenotype.[16,19]

The direction of each blood flow and the corresponding shear stress on ECs can be further partitioned into 3 directions: luminal, interstitial, and transmural (Figure 2D). Luminal flow is responsible for the apical shear stress on the EC surface. The interstitial flow, which originates from the fluid movement within the EC-surrounding tissues, exerts a shearing force on the EC basal side.[20] The transmural flow, which is typically found in the microvasculature and lymphatic vasculature, arises from the pressure difference between the internal and external vasculature and mostly elicits shear forces on EC junctions.[21] These 3 directions of flow patterns play a crucial role in regulating angiogenesis by acting on ECs. At physiological shear stress (≈3 dyn/cm^2), luminal flow has been demonstrated to inhibit VEGF (vascular endothelial growth factor)-induced endothelial sprouting via NO signaling.[22] However, at shear stresses exceeding 10 dyn/cm^2, both luminal and transmural flow stimulate the sprouting of ECs and their subsequent invasion to ECM (extracellular matrix) through MMP (matrix metalloproteinase) 1 induction.[23]

Conversely, both physiological and high-level transmural shear stress universally contribute to the sustained elongation of endothelial sprouts.[23] Notably, the threshold shear stress magnitude for initiating endothelial sprouting is consistent (≈10 dyn/cm^2) between luminal and transmural flow.[23] Furthermore, transmural shear stress has been demonstrated to restore endothelial sprouting following exposure to luminal flow, as evidenced by an in vitro microfluidic bifurcation model.[24] The magnitudes of interstitial shear stress in normal arteries are highly variable due to the presence of various sources in vivo (range, 0.06–0.6 dyn/cm^2), whereas those in atherosclerotic arteries range from 0.33 to 3.27 dyn/cm^2.[25] At physiological levels (0.1–10 μm/s), interstitial flow has been observed to promote endothelial sprouting and angiogenesis against the flow direction, although vascular tubes are in alignment with the applied interstitial flow.[26]

The shear rate of blood influences the magnitude and the pathophysiological effect of shear stress on the endothelium. The shear rate of a shear-thinning fluid (eg, blood) is defined as shear stress/viscosity (Figure 2E).[27] Low magnitudes of shear rate and corresponding shear stress are often observed in larger vessels from the venous system.[28] Meanwhile, the narrowing of blood vessels due to the presence of atherosclerotic plaques alters the magnitudes of shear rate and shear stress. Higher and lower shear stresses are observed in the midstream and downstream portions of plaque, respectively (Figure 2F).[29] At low shear sites, blood viscosity increases due to non-Newtonian characteristics of blood and reversible erythrocyte aggregation,[27] typically resulting in turbulent flow and lower shear stress on EC surface.[30] Low shear rate and shear stress are thought to increase the thrombotic risk, promote endothelial dysfunction, and initiate the development of atherosclerosis.[30] However, at sufficiently low shear rates (<100 s^{-1}), blood displays pseudoplasticity (or shear thinning), a phenomenon whereby blood viscosity is reduced but the shear rate is increased due to erythrocyte disaggregation and deformability. This delays the transition from steady laminar to turbulent flow.[31]

In contrast, high shear rates are associated with low blood viscosity and high shear stress.[28] High shear stress

is generally antiatherogenic but has been linked to plaque destabilization and the formation of vulnerable plaques.[32] High shear rates can have harmful effects on the vasculature, depending on their magnitude and the vascular sites involved. A pathologically high shear rate (>5000 s^{-1}) has been demonstrated to promote the rapid aggregation of platelets, which in turn facilitates thrombus growth.[33] In coronary arteries with advanced atherosclerotic lesions, high shear stress has been shown to promote platelet activation and aggregation, potentially leading to plaque instability and rupture by facilitating fibrous cap erosion.[34] Notably, the majority of in vitro studies investigating the pathophysiological role of shear stress on ECs have used relatively simple Newtonian fluids, such as culture media. This approach reflects a slight deviation from the actual situation in blood, which contains not only blood cells but also a range of blood-borne factors.

The intensities, dynamics, and directional patterns of shear stress exhibit significant variations at different vascular sites, reflecting the intricate complexity of vascular networks and geometries (Figure 2G).[35,36] Arteries with lower shear stress magnitudes often display a higher prevalence of atherosclerotic diseases, as observed in the common femoral artery and superficial femoral artery, with the exception of the supraceliac and infrarenal aorta.[35,36] Additionally, the magnitudes of shear stress exhibit considerable variation among species (Figure 2H). In vivo determination of shear stress intensity by micro-CT (computed tomography) and ultrasound technique revealed that mouse aortic ECs experience a range of shear stress, reaching 600 dyn/cm^2 in <50 ms, ≈2 orders of magnitude higher than that in human aortas.[37] Because mouse ECs indeed experience a much higher shear stress than human ECs in vivo, previous in vitro studies reproducing shear stress from human findings on mouse ECs might lead to inappropriate conclusions.

The intensity of shear stress declines gradually with age. From the second to sixth decade of life, the peak WSS of the common carotid arteries is observed to decrease in both men (from 43 to 26 dyn/cm^2) and women (from 33 to 25 dyn/cm^2; Figure 2I).[38] The age-related decline and impairment in shear stress is associated with a reduction in eNOS (endothelial NO synthase)-mediated NO release,[39] an increased arterial diameter,[38] and the provocation of arterial remodeling.[39] Furthermore, it is possible that aging is associated with increased OSS in specific arteries. In a clinical context, significantly higher OSS was observed in the brachial arteries of older individuals when compared with their younger counterparts.[40]

BIOCHEMICAL EFFECTS OF HEMODYNAMIC SHEAR STRESS

The endothelium, situated between the blood and the surrounding vascular tissues, serves as an interface through which biomechanical stimuli, such as shear stress, are converted into biochemical signals, thereby influencing both ECs and adjacent vascular cells, like vascular smooth muscle cells.[4]

Mechanosensors are located on the apical and basal surfaces and at cell-cell junctions of ECs. They are responsible for transducing biomechanical stimuli derived from shear stress into biochemical signals within ECs (Figure 3). The mechanosensors on the EC apical surface encompass a diverse range of receptors and proteins, including GPCRs (G-protein–coupled receptors), NOTCH1 (neurogenic locus notch homolog protein 1), P2X4 (P2X purinoreceptor 4), PIEZO1 (piezo type mechanosensitive ion channel component 1), and plexin D1. Additionally, mechanosensitive structures, including caveolae, glycocalyx, and primary cilia, are present on the apical surface of ECs.[1] In response to flow-induced stimulations, GPCRs (eg, GPR68 [G-protein–coupled receptor 68]) undergo conformational activation, which in turn triggers calcium influx for mechanotransduction.[41] NOTCH1 maintains junctional integrity, elongates morphology, and mediates alignment with flow in ECs through calcium signaling, thereby eliciting antiatherogenic benefits.[42]

Endothelial PIEZO1, a mechanosensitive calcium channel, elicits antiatherogenic and proatherogenic effects through distinct mechanisms upon LSS and OSS, respectively. LSS has been demonstrated to cause a PIEZO1-mediated calcium influx into ECs and subsequent ATP release. This, in turn, enhances the NO production from activated eNOS through PI3K (phosphoinositide 3-kinase)/AKT (protein kinase B) signaling. In response to OSS, PIEZO1 and G_q/G_{11} coordinate integrin activation, which in turn induces NF-κB (nuclear factor-κB) activation and the initiation of proatherogenic signaling cascades.[43] In response to shear stress, endothelial plexin D1 constitutes a mechanocomplex with VEGFR (vascular endothelial growth factor receptor) 2 and neuropilin-1, which is situated upstream of integrin and junctional complex–mediated mechanotransduction.[44] P2X4, an ATP-operated cation channel, senses LSS to induce calcium influx and upregulate KLF (Krüppel-like factor)-2 for mechanotransduction.[45]

Integrins are present on the EC basal side, where they mediate mechanotransduction.[46] In response to shear stress, integrins undergo conformational activation, which results in a higher affinity toward ECM proteins. The activated integrins are responsible for mediating LSS-related antiapoptotic, antiproliferative, morphological remodeling, and, therefore, antiatherogenic effects through modulating the Rho signaling pathway and YAP (yes-associated protein)/TAZ (transcriptional coactivator with PDZ-binding motif) pathway.[46,47] At cell-cell junctions, mechanosensory complex, comprising PECAM1 (platelet EC adhesion molecule-1), VE-cadherin, and VEGFR2/3, modulates EC alignment, integrin activation, and eNOS activation through PI3K/AKT signaling

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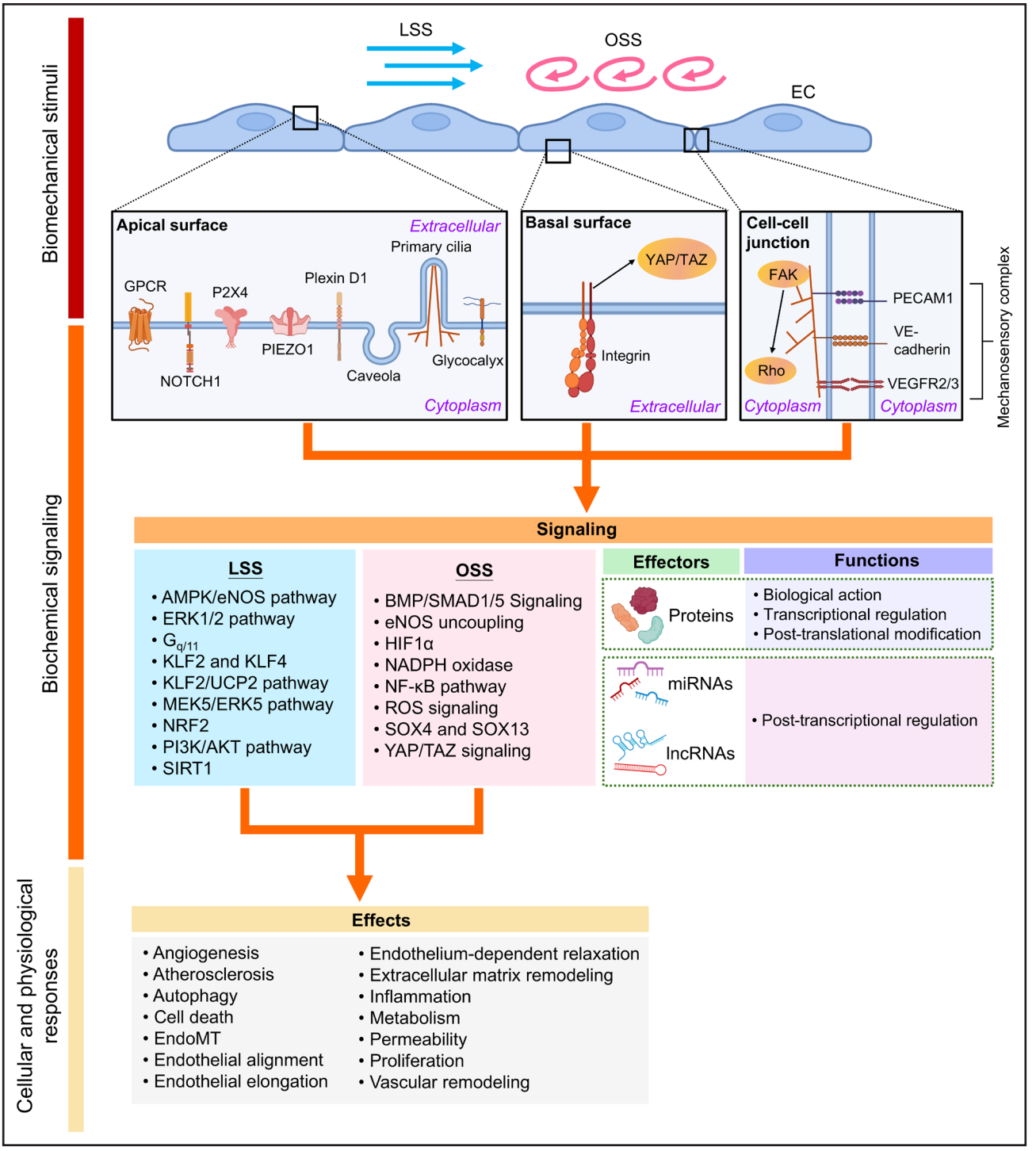

Figure 3. Biochemical effects of hemodynamic shear stress.
Mechanosensors and mechanosensitive structures on the apical and basal surfaces and at cell-cell junctions of endothelial cells (ECs) convert biomechanical stimuli of shear stress to biochemical signals, which modulate endothelial and vascular events through various pathways and the action of different effectors (eg, proteins, microRNAs [miRNAs], and long noncoding RNAs [lncRNAs]). Steady laminar shear stress (LSS) and oscillatory shear stress (OSS) have been shown to activate different pathways, thereby eliciting endothelial and vascular effects. Processing software: Biorender. AKT indicates protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; BMP, bone morphogenetic protein; EndoMT, endothelial-to-mesenchymal transition; eNOS, endothelial NO synthase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GPCR, G-protein–coupled receptor; HIF1α, hypoxia-inducible factor 1α; KLF, Krüppel-like factor; lncRNA, long noncoding RNA; MEK5, mitogen-activated protein kinase kinase 5; miRNA, microRNA; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; NOTCH1, neurogenic locus notch homolog protein 1; NRF2, nuclear factor erythroid 2–related factor 2; P2X4, P2X purinoreceptor 4; PECAM1, platelet endothelial cell adhesion molecule-1; PI3K, phosphoinositide 3-kinase; PIEZO1, piezo type mechanosensitive ion channel component 1; ROS,reactive oxygen soecies; SIRT1, sirtuin 1; SMAD1/5, mothers against decapentaplegic homolog 1/5; SOX, SRY-box transcription factor; TAZ, transcriptional coactivator with PDZ-binding motif; UCP2, uncoupling protein 2; VE, vascular endothelial; VEGFR2/3, vascular endothelial growth factor receptor 2/3; and YAP, yes-associated protein.

in response to LSS.[48] The detailed roles of these mechanosensors have been extensively reviewed by Tamargo et al.[1]

Present on the apical surface of ECs, primary cilia are protrusions consisted of microtubule bundles and connected to the intracellular cytoskeleton. In response to LSS (15 dyn/cm^2), cilia are disassembled and causes cytoskeletal rearrangement.[49] Endothelial cilia sense shear stress to mediate downstream calcium signaling and NO production, where the mechanosensitive function of cilia is dependent on polycystin-1 and polycystin-2.[50] Located on the apical surface of ECs, glycocalyx is a sugar-rich layer, consisted of glycoproteins and proteoglycans. Glycocalyx couples shear stress sensing to calcium signaling, eNOS activation, and NO production.[51] Under physiological LSS, the structure of endothelial glycocalyx remains intact, contributing to enhanced eNOS activity and improved endothelial function. In contrast, pathophysiologically high shear stress disturbs glycocalyx structure, resulting in calcium and sodium influx, suppressed eNOS activity, and alleviated vasodilation.[52]

It should be emphasized that in addition to flow patterns, the magnitudes of shear stress differentially regulate the same mechanosensor/flow-sensitive protein and the downstream signaling cascades. For instance, endothelial PIEZO1 causes calcium influx, promotes vasodilation, and elicits antiatherogenic effect in response to physiological LSS. The further increased LSS due to increased blood flow depolarizes ECs by causing both PIEZO1-dependent calcium and sodium influx, where such depolarization is sufficient enough to be transmitted to surrounding VSCMs to promote vasoconstriction.[53] Although it also triggers calcium influx,[54] OSS exposure at the inner curvature of aortic arch activates the PIEZO1/Gq/G_{11}/integrin cascade to elicit proinflammatory and proatherogenic effects in the vasculature.[43]

Different flow patterns can cause distinct posttranslational modifications of a flow-sensitive protein to alter its molecular role. For example, OSS induces reversible and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase 4–dependent S-oxidation of VEGFR2 at Cys^{1206} to retard VEGF-dependent VEGFR2 activation. Consequently, VEGFR2 S-oxidation promotes endothelial apoptosis and atherogenesis. In contrast, LSS upregulates eNOS to trigger S-nitrosylation of VEGFR2 at Cys^{1206} to facilitate endothelial survival and elicit antiatherogenic effects.[55] It is worthwhile to determine the shear stress ranges that differentiate the molecular roles of various mechanosensors/flow-sensitive proteins upon different flow patterns.

Potential cross talk exists between endothelial mechanosensors upon flow-induced stimulations. As mentioned, disturbed flow causes PIEZO1-mediated and Gq/G11-mediated integrin activation to trigger NF-κB activation in a focal adhesion kinase–dependent manner.[43] Additional to the apical surface, PIEZO1 was also found present at cell-cell junctions to interact with PECAM1. In response to LSS, PIEZO1 is driven to junctions to partner with PECAM1 to mediate calcium-dependent junctional remodeling, while PECAM1 lowers mechanical sensitivity of PIEZO1.[56] PIEZO1 was shown upstream to the proteolytic cleavage of NOTCH1 for its downstream action in LSS-exposed human microvascular ECs.[57] Moreover, P2X4 was found to colocalize with VE-cadherin at cell-cell junctions,[58] though the exact cross talk between P2X4 receptor and the mechanosensory complex remains unclear. Further extensive efforts are needed to unravel the cross talk network among different mechanosensors and mechanosensitive structures in mediating endothelial mechanotransduction.

The mechanotransduction pathways that are activated by shear stress sensing include the ERK1/2 (extracellular signal-regulated kinase 1/2) pathway, the PI3K/AKT pathway, the Rho signaling pathway,[59] and the YAP/TAZ pathway.[47] Activation of mechanotransduction pathways in turn regulates the expression and activity of flow-sensitive TFs (transcription factors), including HIF1α (hypoxia-inducible factor 1α), KLF2, KLF4, NF-κB, and SOX (SRY-box transcription factor) 13, to elicit downstream effects through numerous effector proteins (Table S1). Moreover, shear stress can affect endothelial homeostasis through epigenetic mechanisms involving histone modifications.[60] In addition to protein-coding genes, hemodynamic shear stress also regulates the expression of various noncoding RNAs, particularly microRNAs (miRNAs; Table S2) and long noncoding RNAs (Table S3). In addition to eNOS and NADPH oxidase 4 mentioned,[55] shear stress causes posttranslational modifications through the action of different flow-sensitive enzymes, such as kinases/phosphatases, acetyltransferases/deacetylases, methyltransferases/demethylases, and ubiquitin enzymes (Tables S4 through S7). Further extensive efforts are required to identify novel posttranslational modification sites and corresponding flow-sensitive enzymes in ECs upon flow exposure. The mechanotransduction signaling cascade mediates a range of cellular events in ECs in response to different flow patterns. They include angiogenesis, alignment, cell death, endothelial function, EndoMT, metabolism, inflammation, permeability, and proliferation.

ECs are capable of converting biomechanical stimuli from shear stress into biochemical signals, thereby influencing surrounding cells through paracrine signaling. LSS increases endothelial production of gasotransmitters, including NO and hydrogen sulfide, which causes smooth muscle relaxation and maintains vascular integrity.[61] Through the ERK1/2 and Rho signaling pathways, hemodynamic shear stress modulates endothelial secretion of extracellular vesicles, which are important biological effectors for paracrine signaling.[62] It is important to note that shear stress can regulate the landscape of noncoding RNAs in EC-derived extracellular vesicles.

In a KLF2-dependent manner, LSS upregulates miR-143/145 levels in EC-derived extracellular vesicles, thereby controlling vascular smooth muscle cell phenotypes.[63] Comprehensive profiling of the proteins and noncoding RNAs encapsulated by EC-derived extracellular vesicles in response to different flow patterns shall hold substantial diagnostic, prognostic, and therapeutic values.

Cerebral blood flow generates shear stress, which in turn mediates the function of blood-brain barrier and the inflammation of brain microvascular ECs. In the human cerebrovascular network, physiological shear stress ranges differentially in arteries (10–70 dyn/cm^2) and capillaries (2.8–95.5 dyn/cm^2).[64] Pathologically high shear stress has been demonstrated to disrupt blood-brain barrier integrity through the release of MMP2 and the activation of the Src (tyrosine-protein kinase)/ERK1/2 pathway.[64] In contrast to aortic and venous ECs in noncerebral vasculature, brain microvascular ECs exhibit greater resistance to LSS-induced endothelial elongation, suggesting the presence of unique features that constitute the blood-brain barrier.[65] Despite the presence of mechanosensors (eg, PIEZO1) in cerebral ECs for blood flow control,[66] it remains unclear whether these sensors play a deviated role to maintaining the unique features of the blood-brain barrier.

High WSS initiates cerebral aneurysm, while low WSS at the aneurysm region promotes rupture.[67] High WSS causes endothelial inflammation, subsequent immune cell recruitment, and vascular remodeling, which exacerbate aneurysm development.[68] OSS at the bifurcation of the middle cerebral artery is clinically associated with a higher risk of cerebral aneurysms.[69] It has been demonstrated that OSS induces EndoMT in human brain microvascular ECs through Notch signaling.[70] Additional to WSS magnitudes, comprehensive documentation of the distribution of different flow patterns across different cerebral regions would enhance our understanding of cerebrovascular pathophysiology.

In the liver, liver sinusoidal ECs (LSECs) sense shear stress to modulate hepatic sinusoidal microenvironment and the progression of liver disorders. In other words, LSECs serve as an interface between blood flow and sinusoid. Due to the unique architecture of liver sinusoids, LSECs are generally exposed to nonpulsatile disturbed flow.[71] In healthy individuals, WSS magnitudes of portal veins (10–20 dyn/cm^2) are significantly higher than those from patients with portal hypertension (0–10 dyn/cm^2).[72] Under physiological conditions, shear stress upregulates KLF2 in LSECs to promote the release of vasodilating agents like NO and downregulate the vasoconstrictive molecules like endothelin-1. In an NO-dependent manner, LSECs maintain the quiescence of hepatic stellate cells and a physiological sinusoidal pressure.[71,73]

The progression of chronic liver diseases is multifactorial, associated with long-standing fibrogenesis, microcirculatory dysfunction, altered microarchitecture, and altered hemodynamics. These features potentially contribute to dysfunction and distorted mechanosensing of LSECs, enhancing hepatic stellate cell activation and vasoconstriction. Dysfunctional LSECs lose the capacity to induce vasodilation in response to shear stress, hinder LSEC–hepatic stellate cell cross talk, and increase intrahepatic vascular resistance, aggravating portal hypertension.[71,73] After hepatectomy, the portal flow velocity dramatically increases, greatly elevating shear stress on LSECs. Interestingly, LSECs can sense the elevated shear stress post-hepatectomy to induce liver regeneration through NO release.[73] Previous single-cell study has revealed 3 LSEC subpopulations in cirrhotic mice, where mechanosensitive genes, like *KLF2* and *KLF4*, are downregulated in all subpopulations.[74] Further studies on how different flow patterns alter LSEC heterogeneity are warranted.

Shear stress–mediated mechanotransduction is critical to the regulation of metabolism and phenotypic shift in pulmonary ECs (PECs), where hemodynamic dysregulation correlates to the development of pulmonary arterial hypertension (PAH). In general, PECs are subjected to steady laminar flow in large pulmonary arteries but to a more disturbed flow in pulmonary capillaries, therefore, corresponding to different endothelial phenotypes along the pulmonary vasculature.[75] It was previously shown that WSS magnitudes of main pulmonary arteries from patients with PAH (4.3±2.8 dyn/cm^2) were significantly lower than those of healthy individuals (20.5±4.0 dyn/cm^2).[76]

Physiological flow during normal microvascular perfusion upregulates KLF2, suppresses glycolysis, and improves barrier integrity, corresponding to a vasodilating and quiescent phenotype in PECs. However, during microvascular dysfunction, blood flow becomes disturbed, which enhances hypoxia-mediated HIF-1α activation, glycolysis, and endothelial permeability. Disturbed flow generally causes an inflammatory and prothrombotic phenotype in PECs, potentially aggravating PAH.[77] Additionally, pathologically high shear stress was also shown to promote EndoMT and PAH progression through reducing ETS-family transcription factor.[78] A previous single-cell study revealed endothelial heterogeneity in PAH mouse lungs, where a capillary subpopulation of PECs is particularly associated with a proangiogenic and apoptotic phenotype.[79] Future studies on how different flow patterns modulate PEC heterogeneity are needed.

Placenta represents a distinctive vascular interface, facilitating the transfer of nutrients and gas exchange between the separate maternal and fetal circulations, which display discrete hemodynamic parameters. During pregnancy, hemodynamic adaptations, characterized by elevated cardiac output and expanded blood volume, raise blood velocity and shear stress. This, in turn, promotes vasodilatation in maternal uterine arteries, thereby

increasing placental blood flow.[80] Despite the elevated blood flow rates observed in the maternal circulation, the WSS in the chorionic villi of the maternal placental circulation is <5 dyn/cm^2.[81] In contrast, the estimated WSSs in the umbilical arteries and veins of fetuses at the 32nd and 33rd gestational weeks were 28.1 and 5.2 dyn/cm^2, respectively.[82]

Mechanosensors, such as PIEZO1, are present on fetoplacental ECs to mediate vasodilation through the promotion of eNOS-derived NO production.[83] Gestation has been observed to upregulate endothelial PIEZO1 levels in uterine arteries of pregnant rats.[84] PIEZO1 downregulation in uterine arteries has been postulated to induce vascular dysfunction during preeclampsia.[80] To date, there is still a lack of feasible in vitro models to reproduce the biological characteristics of preeclampsia. Further studies are required to elucidate the expression profiles and detailed roles of different mechanosensors in regulating placental vasoactivity.

One central enigma of hemodynamic research is to understand what factors critically cause distinct mechanosensing, mechanotransduction, and downstream regulations upon different flow patterns. It is generally believed that the spatial and temporal profiles of shear stress, covering its direction, magnitude, frequency, and uniformity, fundamentally determine the biochemical effects of shear stress in ECs. Tremendous efforts have been paid worldwide to investigate the role of flow-sensitive effectors and the mechanisms involved, yet the upstream comprehensive mechanisms of shear stress–induced mechanotransduction remain largely elusive. For example, the threshold shear stress intensities to ignite different endothelial mechanosensors at distinct vascular sites and how the specific structures of different mechanosensors correlate to their differential mechanosensing capabilities remain unclear. Scarce studies have compared the turn-ons and turnoffs of mechanosensors, and the corresponding proportions, in response to different flow patterns.

EXPERIMENTAL MODELS OF HEMODYNAMIC SHEAR STRESS

Biomedical researchers and engineers have developed multiple in vitro experimental models to investigate the mechanistic role of shear stress on cultured ECs under different shear parameters. Meanwhile, 2 principal in vivo experimental models have been used to examine the pathophysiological function of disturbed flow in the development of atherosclerosis (Figure 4).

The parallel-plate flow chamber is widely used for the in vitro study of shear stress (Figure 4A). EC monolayers are cultured on sterilized coverslips and subsequently exposed to a defined shear stress, generated by a computerized pump system within a specified time period. This is achieved by utilizing a flow chamber system to generate LSS and OSS. However, deterioration or mechanical wear of the flow chamber components may lead to liquid leakage, increasing the risk of experimental failure. A cone-and-plate viscometer is another apparatus that is frequently used for hemodynamic studies (Figure 4B). By rotating a Teflon cone around an axis perpendicular to EC-cultured plate, a differential and spatially homogeneous shear stress is applied to ECs. The application of differential shear stress, including LSS, OSS, and even turbulent shear stress, can be achieved through the modification of the cone taper, angular velocity, and rotational patterns of the viscometer.[16]

The orbital shaker approach represents an economical and straightforward method for investigating hemodynamics, whereby culture plates are simply placed on a standard shaker platform (Figure 4C). The shear stress experienced by ECs is dependent on a number of factors, including the angular velocity and orbital radius of the shaker platform, the radius of the culture wells, and the height of the resting culture media. This method generates antiatherogenic flow at the edge and proatherogenic flow at the center of the swirling well, thus allowing the concurrent study of dual profiles of shear stress.[85] However, this method is not suitable for studies that require a uniform shear stress over the entire EC monolayer.

In the past decade, various microfluidic devices have been developed for the rapid and convenient investigation of hemodynamics (Figure 4D). Shear stress can be readily generated by the injection of fluid into microfluidic devices. By varying the route for injected fluid, flow rate, and flow periodicity, microfluidic devices can readily simulate LSS and OSS.[86] Furthermore, it is possible to generate a shear stress gradient for comparative study.[86] Microfluidic devices are particularly suitable for staining and biophysical quantification. Nevertheless, microfluidic devices are not a viable option for a considerable number of biochemical assays due to the restricted number of encapsulated cells. Besides, the in vitro models are unable to simulate 3-dimensional (3D) structure of the vasculature or long-term physiological stimuli (eg, exercise-induced LSS). Notably, it is now possible to study cerebrovascular shear stress in a microfluidic chip model. Mehta et al[87] developed a microfluidic model to biomimic the branching hierarchy of cerebral vasculature for investigating heterogeneous shear stress. Recently, Cherubini et al[88] developed a 3D macrofluidic chip model to mimic the hemodynamic conditions of placental vessels by coculturing human umbilical vein ECs, placental pericytes, and fibroblasts. Advancements in 3D printing and 3D bioprinting will facilitate the development of artificial and biomimetic models, which simulate 3D and multilayered structures of the vasculature.

Due to distinct distributions of WSS in the arterial vasculature, assessing gene and protein expression in disparate sites of normal arteries is a common strategy

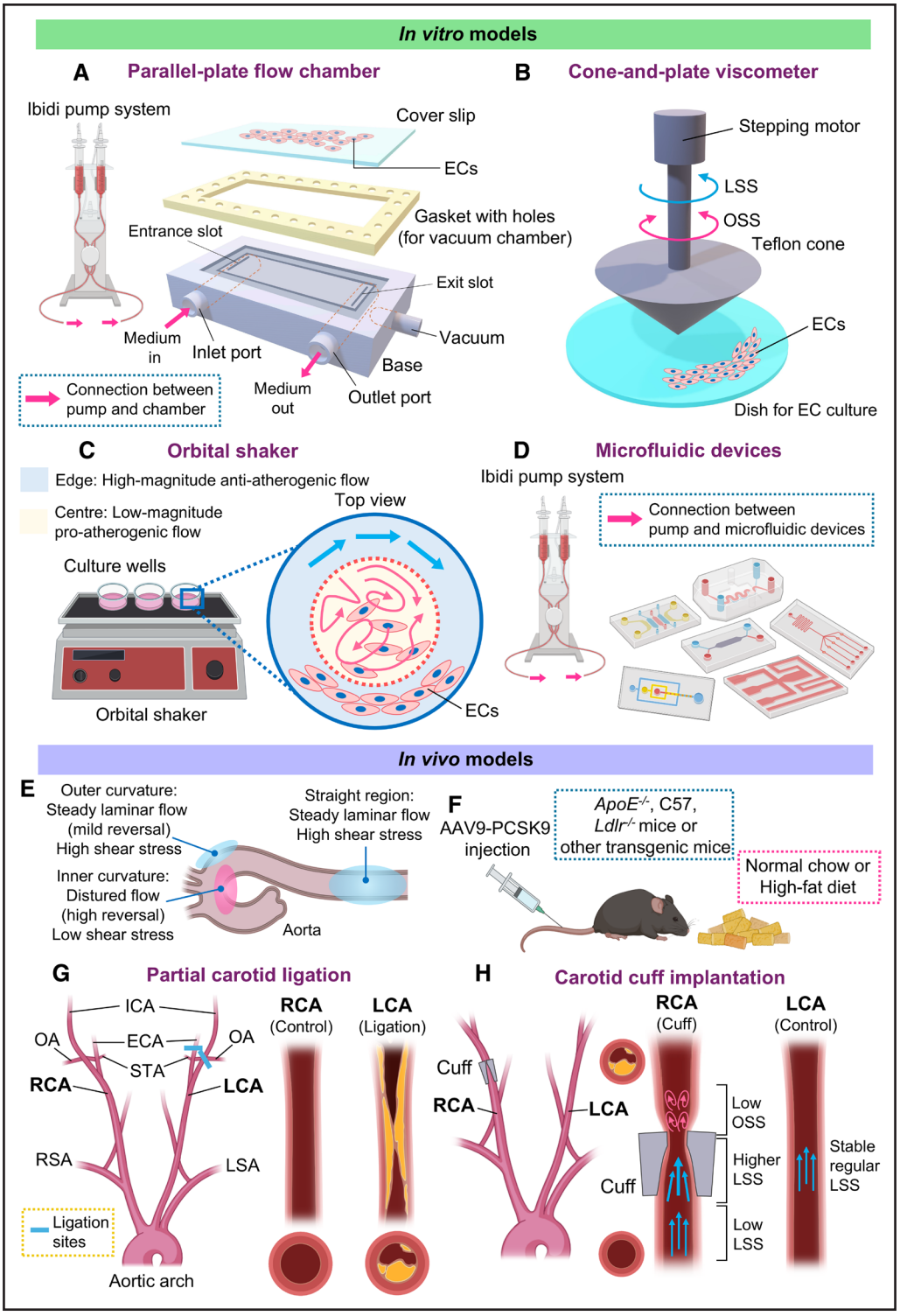

Figure 4. In vitro and in vivo experimental models for hemodynamic study.
A, A parallel-plate flow chamber connected to an ibidi pump system is used to expose endothelial cells (ECs) to differential flow generated by a computer. **B**, Cone-and-plate viscometer, where ECs are exposed to differential flow generated by a rotating Teflon cone. **C**, Orbital shaker, where ECs are concurrently exposed to antiatherogenic and proatherogenic flows. **D**, Microfluidic devices developed for use in a range of hemodynamic studies. **E**, Flow patterns and shear stress magnitudes at different sites of the aorta. **F**, Mouse models commonly used for hemodynamic studies. **G**, Partial carotid ligation model is used to induce disturbed flow in the left carotid artery (LCA) by surgically ligating the external carotid artery (ECA), internal carotid artery (ICA), and occipital artery (OA), while the right carotid artery (RCA) serves as a control. **H**, Carotid cuff implantation model involves the implantation of a constrictive cuff to induce low steady laminar shear stress (LSS), increasing LSS, and low oscillatory shear stress (OSS) along the RCA, while the LCA serves as a control. Processing software: Biorender. AAV9 indicates adeno-associated virus 9; LSA, left subclavian artery; PCSK9, proprotein convertase subtilisin/kexin type 9; RSA, right subclavian artery; and STA, superior thyroid artery.

to investigate the in vivo impact of shear stress (Figure 4E). Steady laminar flow is observed in straight aortic region and outer curvature of the aortic arch, while disturbed flow is dominant in the inner curvature of the aortic arch.[89] Two animal models have been commonly used to study the effect of OSS in the pathogenesis of atherosclerosis through the implementation of surgical procedures in mice (Figure 4F through 4H). The partial carotid ligation (PCL) model entails the surgical ligation of 3 caudal branches (of 4) of the left carotid artery, namely the external carotid artery, internal carotid artery, and occipital artery, while the left carotid artery itself and the right carotid artery (RCA) are left intact. The PCL model in *ApoE*$^{-/-}$ mice, *Ldlr*$^{-/-}$ mice, or high-fat diet–fed mice with PCSK9 (proprotein convertase subtilisin/kexin type 9) overexpression results in OSS with a low magnitude, which consequently causes the rapid formation of atherosclerotic plaques in the entire left carotid artery within 2 to 3 weeks. Meanwhile, the RCA from the same mice serves as an ideal control due to stable flow exposure and the absence of plaque development.[90,91] Consequently, the PCL model is highly suitable for evaluating the role of potential flow-sensitive genes in transgenic mice. Furthermore, the presence of plaques along the entire left carotid artery and the absence of plaques in the RCA allow for the preparation of sufficient samples for omics studies, including bulk, single-cell, and even spatial RNA sequencing.

The carotid cuff model is achieved by implanting a shear stress–altering cone-shaped cuff around an RCA section in Western diet–fed ApoE$^{-/-}$ mice.[92] The cuff implantation causes the generation of 3 shear stress patterns in RCA. LSS with low magnitude, LSS with high magnitude, and OSS with low magnitude are generated in regions upstream to, within, and downstream of the cuff, respectively. Accordingly, the formation of vulnerable plaque, minimal plaque, and stable plaque occurs in regions upstream to, within, and downstream of the cuff, respectively.[92,93] The carotid cuff model is, therefore, particularly suitable for evaluating the effects of differential shear patterns along the same vessel.[93] However, compared with the PCL model, the cuff model, which provides a limited number of samples associated with different shear patterns, might not be suitable for subsequent omics studies.

THERAPEUTIC STRATEGIES TARGETING HEMODYNAMIC SHEAR STRESS

This review addresses the biophysical and biochemical aspects of hemodynamic shear stress, emphasizing its relevance to the development of cardiovascular complications. Targeting shear stress may represent a potential therapeutic strategy against cardiovascular diseases, particularly atherosclerotic diseases. More specifically, the strategies are based on the following concepts: (1) increasing LSS or mimicking its beneficial effects, (2) diminishing OSS or its harmful effects, (3) targeting downstream effectors (eg, flow-sensitive genes and noncoding RNAs) of shear stress, and (4) targeting downstream events of shear stress. This section presents an overview of pharmacological, physiological, surgical, and genetic strategies for targeting shear stress, based on existing preclinical and clinical findings (Figure 5).

LSS and OSS exert differential effects on endothelial homeostasis, influencing NO production, morphology, barrier function, activation, autophagy, plasticity (eg, EndoMT), and cell death of ECs. These 2 shear stress patterns produce opposing effects on superoxide production, inflammation, angiogenesis, and atherogenesis in the vasculature. As previously discussed, shear stress patterns influence these endothelial and vascular events through the mediation of numerous effectors, including genes and noncoding RNAs (Tables S1 through S7). It can be postulated that pharmacological interventions and gene therapies that could partially mimic the beneficial benefits of LSS while mitigating the detrimental effects of OSS are cardioprotective. The core concepts for developing pharmacological and genetic strategies targeting shear stress are to increase the expression and activity of LSS-evoked genetic targets, while decreasing the expression/activity and promoting the clearance of OSS-enhanced genetic targets.

As reviewed by Wang et al,[94] it has been demonstrated that a number of extensively studied cardioprotective agents, including bone morphogenic protein 4 inhibitors, cyclooxygenase-2 inhibitors, glucagon-like peptide 1–elevating agents, KLF2 activators, peroxisome proliferator-activated receptor δ agonists, renin-angiotensin system inhibitors, sirtuin activators, and statins, have the potential to mediate the downstream effectors and events of shear stress, particularly eliciting anti-inflammatory and antiatherogenic effects. It is noteworthy that certain therapeutic agents have the capacity to mimic or accelerate the effects of LSS on endothelial morphology and alignment. For example, resveratrol, an SIRT1 (sirtuin 1) activator, was previously shown to enhance endothelial elongation in static culture and re-endothelialization following arterial injury in vivo.[95] Treatment with tubacin in LSS-exposed (20 dyn/cm^2) ECs has been reported to accelerate endothelial elongation through microtubule acetylation,[96] while microtubule acetylation is necessary for the regulation of endothelial alignment and orientation under physiological shear stress.[96] These highlight the importance of identifying critical regulatory genes and developing more pharmacogenetic strategies to modulate the spatial organization of ECs.

To increase the magnitude of shear stress, one can either pharmacologically elevate the shear rate by increasing cardiac output or decreasing blood viscosity, in accordance with the following formula: shear rate=shear stress/viscosity. In lieu of pharmacological

REVIEW

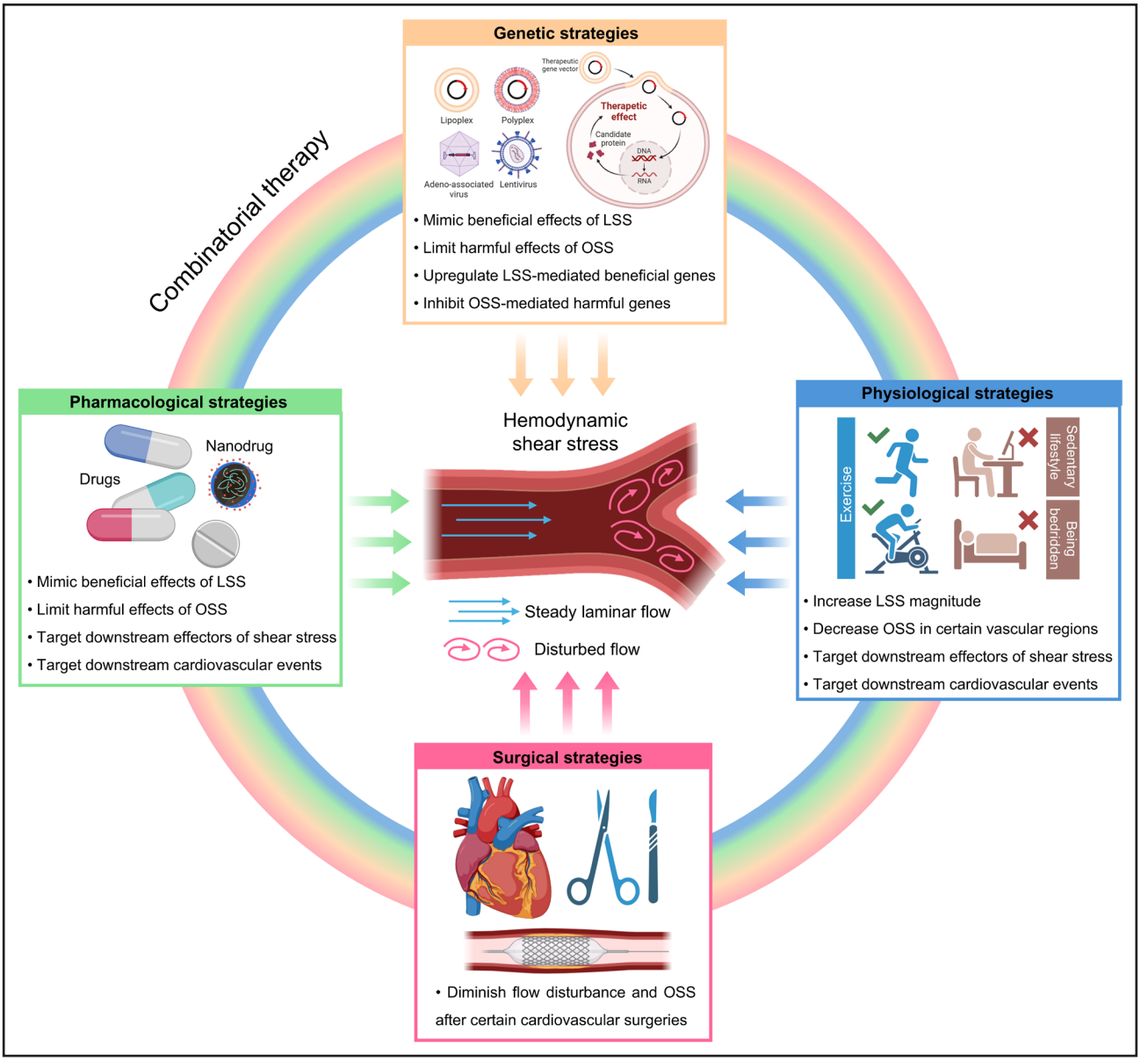

Figure 5. Strategies targeting hemodynamic shear stress.
There are 4 main ways in which pharmacological, genetic, physiological, and surgical strategies can target hemodynamic shear stress. These are (1) increasing steady laminar shear stress (LSS) or mimicking its beneficial effects, (2) diminishing oscillatory shear stress (OSS) or its harmful effects, (3) targeting downstream effectors, and (4) modulating downstream events induced by shear stress. The rational design of combination therapy regimens has the potential to optimize hemodynamic performance. Processing software: Biorender.

elevation of cardiac output by inotropes and reduction of blood viscosity by anticoagulants, which may precipitate adverse cardiovascular effects, it may be prudent to consider increasing cardiac output and lowering blood viscosity in a physiological and healthy manner, with a view of achieving cardiovascular benefits. The presence of numerous risk factors, including obesity, smoking, diabetes, and elevated levels of LDL (low-density lipoprotein), lipoprotein(a), and androgens, has been demonstrated to raise blood viscosity. While not necessarily through the effects of increased shear stress, reducing blood viscosity can mitigate the risks of atherosclerosis and thrombus formation.[27] Ticagrelor, an adenosine enhancer, has been shown to enhance microvascular blood flow by lowering blood viscosity in patients with concurrent lower extremity arterial disease and type 2 diabetes.[97]

In the selection of vectors for drug and gene delivery, it is imperative not to overlook the influence of shear stress on the rate of uptake. Exposure to a convective flow with high shear stress has been demonstrated to inhibit the uptake and transfection activity of lipoplex and adenovirus in cultured ECs, probably due to a reduction in the retention time of the vector on ECs.[98] Nanomaterials are nonviral vectors that are particularly well suited to targeted drug delivery and gene therapy to ECs due to their low immunogenicity. The distribution and uptake of

nanomaterials are influenced by shear stress, with nanomaterials accumulating in vascular regions subjected to low magnitude OSS.[99] The higher the blood flow rates, the lower the ability of ECs to uptake nanomaterials.[100] The careful design of the size, shape, stiffness, and surface attachment of nanomaterials can alter their distribution tendency to LSS or OSS regions. Moreover, shear stress influences the drug release profile of nanomaterials. A lenticular nanovesicle synthesized by artificial 1,3-diaminophospholipid was demonstrated to release contents preferentially upon high shear stress, while maintaining stability at static condition.[101]

It is well documented that exercise is an effective physiological method for increasing cardiac output and reducing blood viscosity. Exercise increases heart rate and cardiac output, resulting in higher shear stress on ECs to improve vascular function. Furthermore, while short-term exercise elevates blood viscosity due to hemoconcentration, regular exercise of long duration gradually reduces blood viscosity in healthy individuals,[102] potentially leading to an increased shear stress at a given blood flow. Nevertheless, it is not feasible for exercise to significantly alter blood viscosity in patients with coronary artery disease and diminished left ventricular function.[102] As the intensity of exercise increases, in terms of maximum heart rate, the blood shear rate and shear stress exerted on ECs also increase. The endothelial shear stress also varies depending on the exercise modality at a given intensity. In general, aerobic exercise causes a higher shear stress than resistance exercise at moderate-to-high intensity. It is worthy to note that high-intensity treadmill exercise was shown to achieve the highest endothelial shear stress (≈84.7 dyn/cm^2) than cycling (≈77.5 dyn/cm^2) and resistance exercise (≈45.6–56.8 dyn/cm^2) in human carotid arteries.[103]

Exercise can enhance LSS, thereby improving vascular function. Furthermore, exercise has been demonstrated to reduce OSS in specific arterial regions, which may contribute to the promotion of vascular benefits. Age-associated increase in retrograde and reciprocating shear in peripheral conduit arteries favors a proatherogenic EC phenotype. Conversely, short-term and steady-state forearm exercise has been demonstrated to reduce OSS in brachial arteries of both young and older healthy individuals.[40] In accordance with the aforementioned findings, chronic endurance exercise ameliorates retrograde and reciprocating shear rates in the common femoral arteries of older individuals.[104] Recently, in silico simulation has indicated that voluntary wheel running significantly increases time-averaged WSS, while simultaneously reducing flow recirculation and OSS in the lesser curvature of mouse aortic arches.[105]

By modifying shear stress patterns, exercise can modulate the expression and activity of flow-sensitive genes and noncoding RNAs, thereby eliciting cardioprotective effects. For example, both LSS on cultured ECs and chronic exercise in diabetic mice have been shown to upregulate the flow-sensitive miR-181b, which attenuates vascular inflammation and oxidative stress.[106] Another study has revealed that exercise enhances vasodilation in diabetic mice through the induction of the KLF2/eNOS axis.[107] Chronic running exercise can epigenetically downregulate NEAT1 (nuclear paraspeckle assembly transcript 1), a flow-sensitive long noncoding RNA, which retards endothelial pyroptosis and atherosclerosis.[108] Further omics and mechanistic studies are required to link exercise-associated cardiovascular benefits to existing flow-sensitive genetic targets. A significant challenge in the development of exercise mimetics is that they primarily target the molecular aspects of exercise, yet are unable to fully replicate the hemodynamic effects associated with exercise.

Venous system is generally characterized by low shear stress, where the average WSS in large veins is often <1 dyn/cm^2 and that in small venules can reach 20 to 40 dyn/cm^2 due to smaller diameters and higher flow rates.[109] Despite the mechanism of chronic venous disease remaining unclear, it is hypothesized that alterations in venous shear stress, venous hypertension, and venous insufficiency collectively aggravate its progression, damaging venous ECs, wall, and valves.[110] Disturbance in hemodynamics and the presence of pathologies in the venous system drive the development of endothelial dysfunction, inflammation, hypoxia, and remodeling in the venous vasculature, which in turn leads to the formation of varicose veins and venous ulcers.[110] The standard physiological means of preventing chronic venous disease include exercise, weight loss, and blood pressure management. Foot exercise and lower limb movements help minimize venous reflux and the exposure of venous ECs to reversing blood flow, by increasing the venous flow rate and facilitating valve closure.[111] However, despite the reestablished pump function of the calf muscle, a chronic exercise regimen did not significantly mitigate venous reflux and improve venous severity scores in patients with advanced venous insufficiency.[112] This suggests that exercise only serves as a supplemental therapy to medical and surgical interventions upon severe venous diseases.

Prolonged periods of sitting and bed rest have been demonstrated to impair cardiovascular homeostasis, resulting in unfavorable hemodynamic changes. Long-term physical inactivity results in increased blood pooling in the lower extremities, leading to augmented arterial pressure and lower shear stress. Therefore, a sedentary lifestyle is associated with an increased risk of endothelial dysfunction and atherosclerotic cardiovascular diseases. Similarly, chronic bed rest due to factors such as aging, injury, and surgery can result in the gradual impairment of vascular function. In a clinical study, resting blood flow and shear rate in femoral arteries were found to be significantly lower in bedridden older individuals than in

nonbedridden young and older counterparts.[113] A comprehensive quantification of hemodynamic parameters in human individuals at different postures would deepen our understanding on hemodynamic pathophysiology.

Cardiovascular surgeries have the potential to induce structural alterations in the vasculature, potentially causing the modifications of hemodynamic patterns. A number of vascular surgical interventions, including balloon and stent angioplasty, and arterial bypass grafting are originally intended to restore blood flow upon vascular occlusion.[114] Certain surgical procedures, such as saphenous vein ablation and stent placement in iliac veins, have been reported to significantly improve the symptoms of chronic venous disease, correct venous reflux, and prevent ulcer recurrence.[115] Nevertheless, these interventions have the potential to create sites of disturbed flow with low shear stress, resulting in the development of atherosclerosis and neointimal hyperplasia. This in turn leads to adverse clinical outcomes, including restenosis after angioplasty, in-stent restenosis, and bypass graft failure, over the long term.[16] As a common surgical intervention to treat narrowing and occlusion of coronary arteries, balloon angioplasty is associated with a high percentage of restenosis occurrence (30%–50%).[116] The altered blood flow with low shear stress following balloon angioplasty promotes restenosis by significantly inducing inward arterial remodeling and vascular smooth muscle cell migration.[117]

Although associated with a lower incidence rate of restenosis (20%–30%),[118] stent placement has been demonstrated to generate a disturbed flow pattern and flow separation on the lateral wall, which in turn amplifies inflammatory cascades, thereby triggering in-stent restenosis.[119] Coronary artery bypass grafting inserts synthetic graft or saphenous vein from patients to create an alternate path for blood flow.[120] However, this intervention can increase the risk of flow disturbance and neointima hyperplasia potentially through the following mechanisms. First, the intervention subjects the inserted vein to the pressure and shear stress of the arterial system, resulting in a biomechanical and geometric mismatch that promotes venous remodeling, disturbed flow establishment, and atherosclerosis.[121] Furthermore, the larger diameter of the inserted saphenous vein in comparison to the grafted coronary artery diminishes blood flow velocity and corresponding shear stress at the grafting region.[122] The presence of venous valves at the grafting region also triggers a disturbed flow pattern. Harvesting saphenous veins without valves could reduce the risk of graft failure.[123]

Notably, certain surgical strategies have been developed to reduce the risk of flow disturbance, neointimal hyperplasia, atherosclerosis, and restenosis following the cardiovascular surgeries mentioned. A surgically created distal arteriovenous fistula (AVF) has been applied to raise blood flow and shear stress above a threshold magnitude. This approach is intended to prevent neointimal hyperplasia and improve graft patency.[124] The insertion of antirestenotic diffuser (Endoart SA) flow divider could elevate shear stress and suppress inflammation in rabbit external iliac arteries, potentially retarding in-stent neointimal hyperplasia.[125] Certain anastomotic patches and cuffs have been applied to counteract the mismatch between coronary artery and venous graft, thus enhancing graft patency through the reduction of disturbed flow.[126] Furthermore, the seeding of EC monolayer at the luminal side of prosthetic vascular grafts, with the application of physiological LSS to enhance EC retention, can significantly improve graft patency.[127] It was previously proposed that the grafts be seeded with genetically modified ECs, which release antithrombotic molecules to enhance graft patency, as a potential gene therapy strategy.[128] With rapid advances in biophysical and biochemical research on cardiovascular hemodynamics, the emergence of more efficacious surgical techniques is anticipated.

The surgical creation of AVFs and the use of synthetic AV grafts for the treatment of chronic hemodialysis are associated with the risks of AVF nonmaturation and neointimal hyperplasia, respectively.[129] It is of particular importance to note that AVF creation results in elevated shear stress values at the anastomosis and the stenosis region.[130] These aberrantly high WSS values have been identified as a key factor in the development of high-risk atherosclerotic plaques, preceded by endothelial inflammation.[131] Despite the advancements made in this field, it is evident that there is still a need for extensive efforts to prevent the adverse effects and improve the hemodynamic performance of surgical procedures. A rational design of combinatorial therapy regimens, involving pharmacological, physiological, and genetic interventions, might optimize hemodynamic performance in the treatment of atherosclerotic cardiovascular diseases.

NEW PERSPECTIVES ON HEMODYNAMIC SHEAR STRESS RESEARCH

For decades, researchers have used a reductionist methodology to elucidate the biochemical functions of hemodynamic shear stress on ECs. In a typical approach, researchers conducted bulk transcriptome sequencing on flow-exposed ECs and vascular tissues to identify potential targets, including genes, miRNAs, and long noncoding RNAs, followed by biological verification and mechanistic study with the aid of pharmacogenetic tools, transgenic animals, and clinical samples. In recent years, the rapid advances and breakthroughs in omics technologies and bioinformatics analysis have enhanced our understanding of the comprehensive regulatory networks of hemodynamic shear stress, in terms of genome, transcriptome, proteome, metabolome, and epigenome.

Furthermore, omics studies can be conducted in an integrated manner at the single-cell level or even in a spatial-temporal landscape. This section presents an overview of the current and future perspectives of omics investigation on hemodynamics and the mechanobiology of ECs (Figure 6).

Numerous single-cell RNA sequencing (scRNA-seq) studies, conducted under disparate experimental settings, have revealed the phenomenon of transcriptional reprogramming in ECs and the existence of EC subpopulations in response to varying hemodynamic stimulations (Table S8). LSS increases the transcriptomic heterogeneity of ECs.[132] Disturbed flow induces proatherogenic, pro-EndoMT, and immune cell–like phenotypes in mouse carotid artery ECs post-PCL.[133] Different shear patterns alter the cellular response and genetic signatures of ECs to antiproliferative drugs, including paclitaxel and rapamycin.[134] scRNA-seq can be applied to identify novel regulators under different flow conditions (eg, KLK10 [kallikrein related peptidase 10]),[135] specific markers of a particular EC subtype (eg, enolase 1 in the EndoMT cluster),[136] and novel flow-sensitive transcription factors (eg, SOX4 and SOX13).[137] It is now feasible to subject EC samples from microfluidic devices (eg, chip systems) to scRNA-seq analysis.[138] Another study conducted scRNA-seq to evaluate the effect of elevated shear stress on rat AVF, thereby providing therapeutic insights into the prevention of AVF failure.[139] Due to its distinct experimental settings used in different scRNA-seq studies, the number of EC subtypes identified upon hemodynamic stimulations varied. In general, shear stress–stimulated ECs can be classified into 3 main subgroups: homeostatic (antiatherogenic, anti-inflammatory, and anti-EndoMT), intermediate, and pathogenic (proatherogenic, proinflammatory, and pro-EndoMT; Figure 6A). Future research is required to unify the findings from disparate scRNA-seq

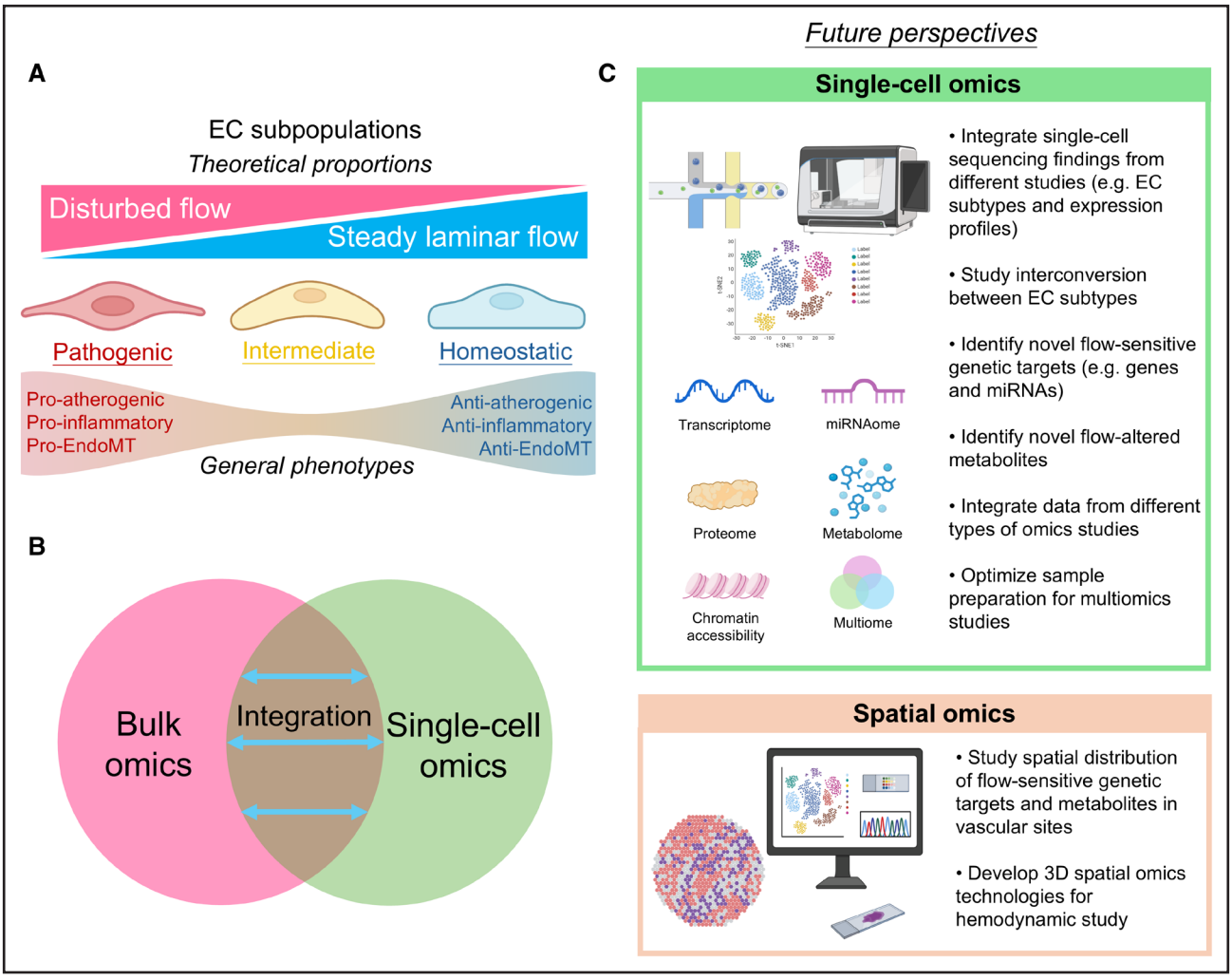

Figure 6. New perspectives for hemodynamic studies.
A, Proposed major endothelial cell (EC) subpopulations and their corresponding phenotypes based on single-cell sequencing results from different hemodynamic studies. **B**, Integration of existing bulk and single-cell omics data as a future perspective for hemodynamic research. **C**, Future perspectives for hemodynamic studies, with a particular focus on single-cell and spatial omics. Processing software: Biorender. 3D indicates 3-dimensional; EndoMT, endothelial-to-mesenchymal transition; and miRNA, microRNA.

studies and confirming the feasibility of interconverting among EC subtypes for therapeutic purposes.

Genome-wide chromatin accessibility can be profiled by bulk assay of transposase accessible chromatin sequencing or single-cell assay of transposase accessible chromatin sequencing, where the latter has the capacity to differentiate open chromatin regions in specific cell types. The coupling of scRNA-seq and single-cell assay of transposase accessible chromatin sequencing enables the concurrent profiling and association analysis of the transcriptome and epigenome of the same individual cells.[140] Previous studies have adopted this approach to distinguish between different EC subtypes[133] and to identify epigenetic and transcriptomic regulation of novel flow-sensitive genes (eg, *KLK10*).[135]

In recent years, significant advances in single-cell proteomics have permitted the detection of thousands of proteins in single mammalian cells through mass spectrometry–based or antibody-based approaches. However, further efforts are needed to improve throughput, sensitivity, and reproducibility. To the best of our knowledge, there is so far no single-cell proteomics analysis on ECs or vasculature upon different hemodynamic conditions. It would be of interest to correlate future single-cell proteomics data with existing scRNA-seq findings on vascular hemodynamics. To date, a plethora of protocols have been devised for single-cell miRNA sequencing, yet there is a dearth of standardization, with its developmental progress significantly lagging behind that of scRNA-seq. It is anticipated that a single-cell miRNA study on flow-exposed ECs will enrich our understanding of miRNA-dependent mechanotransduction.

Bulk multiomics techniques have been used in hemodynamic studies. A previous study conducted bulk RNA sequencing, assay of transposase accessible chromatin sequencing, and histone chromatin immunoprecipitation followed by sequencing on pulsatile shear stress–exposed ECs.[141] However, despite the affordability of bulk multiomics, these techniques present limited information regarding cellular heterogeneity. Single-cell multiomics approaches are designed to profile cellular heterogeneity by integrating multiple single-modality omics techniques, thereby facilitating a comprehensive understanding of the signaling network induced by flow patterns. The primary challenge is the preparation of an adequate amount of endothelial or vascular samples to prevent data loss, given the limited coverage of each omics layer on the multiomics platform. As an alternative, one may consider the application of computational analysis for the integration of single-modality omics data from discrete hemodynamic studies of bulk or single-cell sequencing.

Spatial omics represents a novel frontier in biomedical research, offering the potential to integrate specific tissue compartments and regions with their associated molecular characteristics (eg, transcript and protein abundance) in a simultaneous manner. This approach promises to facilitate more profound biological insights. Previous studies have used bulk or single-cell sequencing on ECs or vascular samples subjected to disparate flow patterns, separately, thereby providing only limited spatial genetic insights. Future spatial omics studies have the potential to facilitate transcriptomic or proteomic profiling at disparate sites (eg, branches, inner-outer curvatures, and internal-external vasculature) within the same vessel. The prospective advancement of spatial multiomics and 3D spatial omics will facilitate the acceleration of hemodynamic research.

Previous studies have used bulk metabolomics to identify potential metabolic reprogramming of ECs in response to hemodynamic stimulation. For example, an integrated metabolomic and proteomic study demonstrated that LSS downregulates cholesterol metabolism and alters LDLR (LDL receptor) glycosylation in cultured ECs.[142] Another untargeted metabolomic study indicated that wheel running exercise activates endothelial stearoyl-CoA desaturase 1, which catalyzes oleic and palmitoleic acids for endothelial protection in mice through the mitigation of flow recirculation.[105] Future advances in single-cell metabolomics will permit detailed profiling of flow-altered metabolites in single ECs and their encapsulated organelles. Meanwhile, spatial metabolomics will expedite the rapid identification of more flow-sensitive metabolites and their spatial distributions (eg, branches and curvatures) along the vasculature.

CONCLUDING REMARKS

This review article revisits the biophysical and biochemical roles of hemodynamic shear stress on ECs in the context of cardiovascular pathophysiology. The intensity and dynamics of shear stress are dependent on a number of factors, including vascular localization, species, temporal control, and aging. Furthermore, shear stress plays a crucial role in maintaining the homeostasis of cerebral and placental vasculature. A plethora of in vitro and in vivo experimental models have been used in the mechanistic study of shear stress, yet they are associated with certain limitations. Pharmacological, physiological, surgical, and genetic strategies that could target shear stress may represent potential therapeutic options against cardiovascular diseases. The recent advances in omics technologies will facilitate hemodynamic studies to a significant extent. It is anticipated that future multiomics, single-cell multiomics, metabolomics, and spatial omics studies will facilitate a more comprehensive understanding of the regulatory network mediated by hemodynamic shear stress.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S8

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