Cells in the tissues and organs of a living body are subjected to mechanical forces, such as pressure, friction, and tension from their surrounding environment. Cells are equipped with a mechanotransduction mechanism by which they perceive mechanical forces and transmit information into the cell interior, thereby causing physiological or pathogenetic mechano-responses. Endothelial cells (ECs) lining the inner surface of blood vessels are constantly exposed to shear stress caused by blood flow and a cyclic strain caused by intravascular pressure. A number of studies have shown that ECs are sensitive to changes in these hemodynamic forces and alter their morphology and function, sometimes by modifying gene expression. The mechanism of endothelial mechanotransduction has been elucidated, and the plasma membrane has recently been shown to act as a mechanosensor. The lipid order and cholesterol content of plasma membranes change immediately upon the exposure of ECs to hemodynamic forces, resulting in a change in membrane fluidity. These changes in a plasma membrane's physical properties affect the conformation and function of various ion channels, receptors, and microdomains (such as caveolae and primary cilia), thereby activating a wide variety of downstream signaling pathways. Such endothelial mechanotransduction works to maintain circulatory homeostasis; however, errors in endothelial mechanotransduction can cause abnormalities in vascular physiological function, leading to the initiation and progression of various vascular diseases, such as hypertension, thrombosis, aneurysms, and atherosclerosis. Recent advances in detailed imaging technology and computational fluid dynamics analysis have enabled us to evaluate the hemodynamic forces acting on vascular tissue accurately, contributing greatly to our understanding of vascular mechanotransduction and the pathogenesis of vascular diseases, as well as the development of new therapies for vascular diseases.

Keywords: endothelial cell, shear stress, stretching tension, mechanobiology

Introduction

Blood vessels are not simply the conduits through which blood passes but active organs that sense and respond to changes in environmental conditions, such as blood flow, blood pressure, oxygen, and various metabolic substances, thereby maintaining the homeostasis of the circulatory system in the living body. Endothelial cells (ECs) lining the inner surface of blood vessels play a central role in the adaptive responses to such environmental changes. ECs act as a barrier that prevents substances in the blood from freely permeating vascular tissues, and they also perform a variety of functions that are essential for the body. For example, ECs regulate blood pressure and the distribution of blood flow to tissues and organs by releasing many types of bioactive substances that alter the tonus of blood vessels. They produce proteins involved in blood coagulation and fibrinolysis to protect against thrombus formation in the blood vessels and secrete cell growth factors that work in angiogenesis and vascular remodeling. In addition, ECs are intimately involved in tissue inflammation and immune responses by interacting with leukocytes via adhesion molecules and cytokines. These diverse functions of ECs are regulated not only by chemical mediators, such as hormones, autacoids, and neurotransmitters, but also by hemodynamic forces based on blood flow and blood pressure.1 Until now, many studies have gradually revealed the mechanism of mechanotransduction, by which ECs sense hemodynamic forces and transmit their information into the cell interior, leading to cellular...
responses that include changes in cell morphology, function, and gene expression. Under physiological conditions, the responses of ECs to hemodynamic forces work to maintain circulatory homeostasis. Under pathogenetic conditions, however, these responses do not work properly, leading to the development of various vascular diseases, such as hypertension, thrombosis, aneurysms, and atherosclerosis. Here, we review the mechanotransduction of hemodynamic forces in ECs and its roles in the pathogenesis of vascular diseases.

Hemodynamic Forces Acting on Vascular Cells

ECs are constantly subjected to fluid shear stress generated by flowing blood, as well as stretching tension and transmural pressure produced by blood pressure. The intensity of shear stress (τ) is calculated as τ = 4μQ/πr³ (where μ is the blood viscosity, Q is the blood flow volume, π is the circumference ratio, and r is the radius of the blood vessel). Under physiological conditions, ECs are exposed to shear stresses ranging from 10 to 20 dyne/cm² in the aorta and from 1 to 6 dyne/cm² in the veins. Since blood periodically flows in both direction and intensity, shear stress also increases and decreases within a single heartbeat. Blood flow in the linear part of the artery is laminar, whereas blood flow in the curved and bifurcated parts of the artery is a disturbed flow with stagnation, recirculation, and vortices. The degree of stretching under physiological conditions is around 9%–12% in the human aorta, 1%–2% in the carotid artery, 2%–15% in the femoral artery, and 6%–10% in the pulmonary artery.6

Endothelial Mechanotransduction

When hemodynamic forces act on ECs, their information is perceived and converted into biochemical signals via mechanosensors and transmitted into the cell interior. A characteristic feature of the mechanotransduction of hemodynamic forces is the almost simultaneous activation of a variety of mechanosensors and their downstream signaling pathways. As a result of mechanotransduction, intracellular metabolic activities change, and the expression of many genes is altered via the activation of transcription factors and mRNA stabilization, leading to changes in cell morphology and function as cellular responses to hemodynamic forces. A number of previous studies have shown that a variety of mechanosensors act in ECs, including membrane molecules (such as ion channels, receptors, adhesion molecules, and glycoproteins), membrane microdomains (such as primary cilia and caveolae), plasma membranes, and the cytoskeleton (Fig. 1).

Here, we describe mechanosensors that mainly respond to physiological shear stress generated by laminar flow. The mechanosensors that detect pathogenetic shear stress caused by disturbed flow have not been fully elucidated. However, disturbed flow with stagnation, recirculation, and vortices typically occurs at bifurcations and curvatures of blood vessels and can be caused by vascular stenosis, aneurysms, and endovascular stents.

Mechanosensors

Membrane molecules

Hemodynamic forces are known to open many types of ion channels, including K⁺ channels, Cl⁻ channels, as well as transient receptor potential (TRP) and Piezo channels that transfer Ca²⁺. Some ion channels are also activated secondarily by hemodynamic forces. For example, shear stress releases adenosine triphosphate (ATP) from ECs,12–13 which opens ATP receptors P2X and P2Y that are present in the cell membrane, allowing the influx of extracellular Ca²⁺ into the cell and Ca²⁺ release from the endoplasmic reticulum to the cytoplasm.14–15 Recently, shear stress was shown to increase ATP production immediately by augmenting mitochondrial oxidative phosphorylation, which in turn leads to ATP release from ECs.16

Hemodynamic forces also activate various types of membrane receptors in a ligand-independent manner, including tyrosine kinase-type receptors, such as vascular endothelial growth factor receptor (VEGFR),17 platelet-derived growth factor receptor (PDGFR),18 and angiopoietin receptor Tie-2,19 and guanosine triphosphate (GTP)-binding protein coupled receptors (GPCRs).20

Integrins are transmembrane proteins that function mechanically by attaching the cell cytoskeleton to the extracellular matrix (ECM). When ECs are exposed to hemodynamic forces, integrins sense changes in tension and transmit them to the cytoskeleton through focal adhesion, which consists of talin, vinculin, paxillin, and α-actinin.21

The application of stretching tension to cell surface β1 integrins results in the almost immediate activation (< 4 ms) of Ca²⁺ influx through transient receptor potential vanilloid 4 (TRPV4) channels within the focal adhesion in ECs.22 Platelet endothelial cell adhesion molecule-1 (PECAM-1), a cell-to-cell adhesion protein, is phosphorylated in response to shear stress, thereby transducing the biomechanical force into downstream biochemical signals through the activation of small G protein Ras kinase and extracellular signal-regulated kinase (ERK).23 In addition, vascular endothelial (VE)-cadherin, an intercellular adhesion molecule, is connected to the cytoskeleton via β-catenin and plakoglobin in the cell, and VE-cadherin has been shown to form a complex with PECAM-1 and VEGFR to act as a mechanosensor of shear stress.24
The vascular endothelial surface is covered by a layer of proteoglycans. When proteoglycans are subjected to shear stress, they alter their structure and transmit the biomechanical force into the cell through changes in the local concentrations of ions, amino acids, and growth factors that proteoglycans contain or by exerting tension on the cytoskeleton linked to the intracellular domains of proteoglycans.\textsuperscript{25}

**Membrane microdomains**

Primary cilia are rod-like organelles protruding from the apical cell membranes and connected through the microtubule organizing center to cytoplasmic microtubules. They have been shown to function as mechanosensors for fluid shear stress.\textsuperscript{26} When primary cilia are bent downstream by fluid flow, their deformation transmits shear stress signals into cells through microtubules and also activates Ca\textsuperscript{2+}-permeable ion channels, as well as triggers Ca\textsuperscript{2+} signaling. Two transmembrane proteins, polycystin (PC)-1 and PC2 are members of a superfamily of TRP channels and are localized on the cilia; together they are involved in shear stress-induced Ca\textsuperscript{2+} influx.\textsuperscript{27} ECs in which PC1 and PC2 have been knocked out are unable to transduce shear stress into changes in the intracellular Ca\textsuperscript{2+} concentration.\textsuperscript{28}

Caveolae are flask-shaped invaginations (50–100 nm in length) of the cell membrane; since they contain many...
receptors, ion channels, and signaling factors, they are known to act as a platform by which external information can enter cells. When hemodynamic forces are applied to the cell, the caveolae’s component proteins, caveolin and cabin, move, and the structure of the caveolae changes, resulting in the activation of various ion channels and receptors distributed in these areas and leading to the activation of downstream signaling pathways.

**Cytoskeleton and plasma membrane**

Cytoskeleton components, such as actin filaments, intermediate filaments, and microtubules, are responsible for holding cells in their shapes. A cell can maintain a certain shape because the cytoskeleton is in mechanical balance, which is known as a tensegrity (tension integrity) model. When hemodynamic forces are applied to a cell, the cytoskeleton perceives changes in tension and undergoes rearrangement, which directly activates signaling molecules associated with the cytoskeleton.

Plasma membranes are continuous bilayers of phospholipids in which various lipids and proteins are assembled. Plasma membranes are fluid so that lipids and proteins can move rapidly in a lateral direction. The phospholipid phase (lipid order) of the plasma membrane differs from one place to another, and two distinct lipid order states, a liquid-ordered (Lo) state and a liquid-disordered (Ld) state, can coexist in the plasma membrane. In a liquid-ordered state, the alkyl chains of fatty acids are regularly oriented and their movement is relatively restricted, while in a liquid-disordered state, the orientation of the alkyl chains is disrupted and their flexion movement is active. The transition between these two lipid order states is known to occur depending on a variety of factors, including changes in lipid composition, cholesterol, water content, ion concentration, temperature, and pH. The physical properties of plasma membranes, such as fluidity and lipid order, have recently been found to change in response to hemodynamic forces. Shear stress increases the fluidity of plasma membranes and shifts their lipid order from a liquid-ordered state to a liquid-disordered state, while stretching tension causes the opposite reaction in terms of both fluidity and lipid order. These phenomena also occur in the artificial lipid bilayers of liposomes, which are composed of phospholipids and cholesterol, indicating that these physical phenomena occur independently of cellular receptors, cytoskeletons, and metabolic activities. The fact that the physical properties of plasma membranes change in opposite directions in response to shear stress and stretching tension indicates that plasma membranes can differentiate shear stress and stretching tension as different mechanical forces. More recently, shear stress has been found to decrease the cholesterol content of plasma membrane immediately. Since membrane cholesterol is known to affect the activities of many types of ion channels and receptors expressed in the plasma membrane, the hemodynamic force-induced alteration in membrane cholesterol dynamics might be a trigger for mechanotransduction in ECs. These findings suggest the existence of a new mechanism of mechanotransduction, in which changes in the physical properties of the plasma membrane occur upstream of mechanotransduction that, in turn, changes the conformation and function of the molecules and microdomains present or bound to the plasma membrane, thereby activating downstream signaling pathways.

**Vascular Diseases Related to Hemodynamic Forces**

**Hypertension**

The physiological range of shear stress stimulates the production of various smooth muscle relaxants in ECs and, conversely, inhibits the production of smooth muscle constrictors, thereby promoting vasodilation and decreasing blood pressure. An increase in blood flow causes acute vascular dilation, which is mainly due to the release of nitric oxide (NO), a potent smooth muscle relaxant, from ECs. When cultured ECs are subjected to shear stress in a flow-loading device, ECs increase NO production in a shear-stress-intensity-dependent manner. This increase in NO production is based on the activation of endothelial NO synthase (eNOS) and an increase in eNOS gene expression. Shear stress increases intracellular Ca\(^{2+}\) concentrations and activates protein kinases and cofactor tetrahydrobiopterin (BH4), all of which lead to the activation of eNOS. In ECs cultured from mice, of which purinoceptor 4 (P2X4), an ATP-gated ion channel, was knocked out, neither the shear-stress-induced increase in the intracellular Ca\(^{2+}\) concentration nor NO release occurred, resulting in a significant elevation in blood pressure. Shear stress also increases the gene expression of eNOS through the binding of transcription factor nuclear factor-kappa B (NF\(\kappa\)B) to the GAGACC (shear stress response sequence) in the promoter of the eNOS gene and the stabilization of eNOS mRNA via polyadenylation. In addition to NO production, shear stress increases the production of other smooth muscle relaxants, including prostacyclin (prostaglandin I2 [PGI2]), C-type natriuretic peptide (CNP), and adrenomedullin.

Shear stress also decreases angiotensin-converting enzyme expressed on EC membranes that convert angiotensin I into a potent vasoconstrictor angiotensin II, which in turn prevents blood pressure from increasing.

In vivo, shear stress, and stretching tension act simultaneously on the vascular endothelium. Changes in the gene expression of eNOS, a vasodilator, and endothelin (ET), a vasoconstrictor, were analyzed in ECs that were cultured in an elastic silicone tubes and subjected to shear stress and stretching tension either alone or simultaneously. Cyclic stretching alone had no effect on the eNOS mRNA levels, whereas shear stress alone resulted in an increase in the eNOS mRNA levels. The concomitant application of the two forces increased the eNOS mRNA levels similar to that
produced by shear stress alone. On the other hand, cyclic stretching alone resulted in an increase in the ET mRNA levels, whereas shear stress alone resulted in a decrease in the ET mRNA levels. When ECs were exposed to the two forces simultaneously, no significant change was seen in the ET-1 mRNA levels, suggesting that the opposing effects of each force alone cancelled each other when the forces were applied together. Thus, vascular tonus seems to be regulated by the combined effects of blood flow and blood pressure on ECs.

**Thrombosis**

Laminar shear stress acts to increase the antithrombotic activity of ECs. Both NO and PGI2, the production of which is increased by shear stress, have potent inhibitory effects on platelet aggregation. Thrombomodulin, a glycoprotein expressed on the EC surface, not only prevents thrombin from causing fibrinogen coagulation and platelet aggregation but also activates protein C, which inactivates blood clotting factors. Shear stress increases the expression of thrombomodulin at both the protein and gene levels in ECs. Shear stress also increases heparan sulfate proteoglycans that are expressed on the surfaces of ECs and have an anti-blood coagulation action. On the other hand, ECs secrete plasminogen activator (PA), which is involved in the production of plasmin that dissolves fibrin, and shear stress stimulates ECs to produce PAs. If these effects of shear stress are impained, thrombi are more likely to form. For example, one possible cause of economy class syndrome, a thrombosis that occurs after prolonged sitting in an airplane, is a reduction of blood flow in the veins of the lower extremities, impairing the enhancing effects of shear stress on the antithrombotic activities of ECs. Platelets, as well as ECs, are known to respond to changes in hemodynamic forces. When the lumen of a blood vessel is partially obstructed by an atherosclerotic plaque or an intra-vascular device or when the geometry of a blood vessel is greatly altered due to an aneurysm, blood flow perturbations occur, enhancing the development of shear microgradients. When platelets flow through these areas, they are exposed to shear microgradients that contain a shear acceleration phase, a peak shear phase, and a shear deceleration phase, and they tend to aggregate and adhere to exposed thrombogenic surfaces, resulting in the formation of thrombi.

**Aortic and Cerebral Aneurysms**

Aneurysms, which are localized dilatations of arteries, can occur as a result of atherosclerosis, genetic degenerative diseases such as Marfan syndrome, inflammatory diseases such as Takayasu arteritis and Behcet’s disease, age-related changes in the arteries, infections, and hypertension. Hemodynamic forces play a major role in the formation and development of aneurysms. In fact, ligation of the common carotid artery on one side that increases blood flow to the contralateral carotid artery and simultaneous ligation of a branch of the real artery that induces hypertension have been shown to cause cerebral aneurysms at a high frequency in mice and rats. In humans with cerebral aneurysms, the blood flow velocity in the internal carotid artery has been observed to be significantly greater on the side of the aneurysm. Aneurysms occur in the locations of high shear stress. Once an aneurysm is formed and the shape of the blood vessel changes, the magnitude and distribution of the shear stress and stretching tension on the vessel wall change, leading to the expansion of the aneurysm and, in some cases, its rupture.

Computational fluid dynamics (CFD) and 4D-flow MRI have revealed the details of the hemodynamics around and within aneurysms. Blood flow patterns within aneurysms are highly complex, with secondary flows such as recirculation, stagnation, and detachment, in addition to being 3D, with swirling flows and vortices. These disturbed flows apply unsteady shear stresses on ECs within aneurysms, and these stresses vary in magnitude and direction over time. In a CFD simulation of an aneurysm model, shear stress was shown to decrease within the aneurysm and to become one-tenth of the shear stress at the center of the aneurysm and double the shear stress near the downstream outlet of the aneurysm; in other words, a very large shear stress gradient was observed.

The histological features of aneurysms include the accumulation of inflammatory macrophages and the excessive degradation of extracellular matrices. The transcription factor NFκB plays a central role in the accumulation of macrophages, and shear stress is involved in the activation of NFκB. In a rat model of cerebral aneurysm, chronic inflammation involving the activation of NFκB in ECs and subsequent macrophage infiltration was shown to lead to a weakening of the cerebral vessel wall and the formation of an aneurysm. Shear stress increases the production of prostaglandin E2 (PGE2) via cyclooxygenase-2 (COX-2), and its receptor-mediated signaling is responsible for NFκB activation. Indeed, the application of shear stress to cultured ECs activates NFκB. A low and disturbed shear stress also reportedly causes the sustained activation of NFκB and the accumulation of macrophages in the carotid artery endothelium of mice. The activation of NFκB in ECs leads to an increase in vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1), which are involved in the adhesion and migration of lymphocytes, respectively, and lymphocytes entering the vascular tissue release cytokines to activate macrophages and induce inflammation. The critical role of inflammation in aneurysm formation is illustrated by the fact that experimentally induced intracranial aneurysms were significantly suppressed in both MCP-1-deficient mice and lymphocyte-deficient mice.

In the tissue of aneurysms, extracellular matrices such as collagen and elastin, which are necessary to maintain the
strength of the vascular tissue, are reduced. A decrease in collagen causes fragility of the vascular tissue, leading to the rupture of the arterial wall, and a decrease in elastin leads to decreased elasticity and easy extensibility of the aneurysm wall. Since the production of collagen by ECs is promoted by shear stress, a decrease in shear stress leads to a decrease in the amount of collagen in the aneurysm tissue. On the other hand, higher than physiological levels of shear stress increase the production of matrix metalloproteinases (MMPs), such as collagenase, and promote the degradation of the extracellular matrices of the vessel wall. After being secreted, MMPs are bound to the extracellular matrix in an inactive state but are activated by PA. Since shear stress augments the production of tissue-type PA (tPA) from ECs, collagenase is thought to be activated more strongly in the aneurysm outlet wall, where a high shear stress is present.

In this way, the endothelial mechanotransduction of shear stress mediates tissue inflammation and weakens the blood vessel wall, leading to aneurysm formation, progression, and rupture. Concerning the role of blood flow in aneurysms, ECs respond not only to the intensity of shear stress but also to its temporal and spatial gradients, and differences in flow characteristics, such as turbulent versus laminar flow, must also be taken into account. Furthermore, although this review has focused mainly on the responses of ECs to blood flow, the responses of both smooth muscle cells in the tunica media and fibroblasts in the adventitia to stretching tension and transmural pressure produced by changes in blood pressure also play critical roles in the pathogenesis of aneurysms.

**Atherosclerosis**

Atherosclerotic lesions occur at specific sites of curvature and bifurcation in relatively large arteries. At these locations, not only is the blood flow slow but secondary flows, such as recirculation, detachment, and vortexes, are also present. Based on these facts, hemodynamic forces, especially disturbed flow, have been thought to play important roles in the development and progression of atherosclerotic lesions. Atherosclerotic lesions, similar to aneurysms, are mainly the result of chronic inflammation. During the process of atherosclerotic plaque formation, leukocytes and lipids in blood enter into the subendothelium, and monocytes, a type of leukocyte, differentiate into macrophages. These macrophages release cytokines, causing inflammation and phagocytose lipids, becoming foam cells. During this process, smooth muscle cells are transformed from a contractile type to a synthetic type, then migrate and proliferate, resulting in a thickening of the intima and a narrowing of the vessel lumen.

In the early stages of plaque formation, leukocytes in the bloodstream first adhere to ECs to enter into the subendothelium. This adhesion takes place through the specific binding of adhesion molecules expressed on the cell membranes of both cells. As blood flow has the effect of physically detaching leukocytes from ECs, the adhesion of leukocytes to ECs occurs where blood flow velocity is slow and the shear stress is less than 2 dynes/cm². Shear stress also affects leukocyte adhesion to ECs through the expression of adhesion molecules on ECs. When cultured ECs were subjected to laminar shear stress, their expression of VCAM-1 decreased in a shear-stress-intensity-dependent manner and the number of lymphocytes adhering to the ECs decreased significantly. Conversely, shear stress generated by disturbed flow increased the expression of VCAM-1 and E-selectin, an endothelial-leukocyte adhesion molecule. Thus, leukocyte adhesion to ECs and migration into the subendothelium were more likely to occur under disturbed flow. In addition, disturbed flow promotes inflammation by activating NFkB and producing MCP-1 in ECs whereas laminar flow activates Krüppel-like factor 2 (KLF2), a transcription factor that plays a crucial role in maintaining normal EC function. KLF2 acts in an anti-inflammatory and athero-protective manner by suppressing the cytokine-induced expression of VCAM-1 and E-selectin in ECs.

In atheromatous plaques, low-density lipoprotein (LDL) accumulates in the subendothelium, but its molecular mechanism is not yet well understood. It has been hypothesized that LDL passes through leaky junctions in the endothelium, which are created when EC proliferation is locally increased and old and new cells are replaced. Laminar flow suppresses EC proliferation, whereas disturbed flow has a stimulating effect on EC proliferation. Disturbed flow is also known to increase the protein permeability of the vascular endothelium. As for reactive oxygen species (ROS) involved in lipid oxidation, disturbed flow activates nicotinamide adenine dinucleotide (NADH)-oxidase and increases ROS production whereas laminar flow increases both superoxide dismutase (SOD), which inactivates ROS, and heme oxygenase-1 (HO-1), which inhibits lipid oxidation. Therefore, lipid oxidation and permeation into the subendothelium are more likely to occur at sites where disturbed flow with unsteady and relatively low shear stress acts.

In atheromatous plaques, intimal thickening occurs with the migration and proliferation of smooth muscle cells. ECs produce NO, PGI₂, CNP, and adrenomedullin, which inhibit smooth muscle cell proliferation, and laminar flow has the effect of increasing these factors. On the other hand, disturbed flow stimulates the production of platelet-derived growth factors (PDGF-A, -B) and ROS, which stimulate smooth muscle cell migration and proliferation. The urokinase-type PA (uPA) produced by ECs not only has a fibrinolytic activity but also degrades the extracellular matrix in the vascular wall and stimulates smooth muscle cell proliferation via uPA receptors. The protein and gene expression levels of uPA in cultured ECs increase in response to disturbed flow, while they decrease in response to laminar shear stress. In fact, the increased expression of uPA has been
observed in vivo in the endothelium, smooth muscle, and macrophages of atheromatous plaques. At sites where disturbed flow acts, intimal thickening can occur through a combination of its own effect and the loss of the effect of laminar flow. Thus, disturbed flow is thought to be pro-atherosclerotic, whereas laminar flow is athero-protective.

**Conclusion**

Vascular diseases, such as hypertension, thrombosis, aneurysms, and atherosclerosis, are caused by a complex combination of genetic background and a number of acquired environmental factors, including lifestyle, hyperglycemia, dyslipidemia, ageing, and hemodynamic factors. In this context, hemodynamic factors, such as blood flow and blood pressure, play a notable role. In particular, the EC mechanotransduction of hemodynamic forces plays crucial roles in the formation and progression of both aneurysms and atherosclerotic lesions. Recent rapid progress in detailed imaging techniques and the CFD analysis of vascular geometry and blood flow in vivo have made it possible to assess the shear stress acting on the local vascular endothelium accurately. Such information is expected to contribute greatly to our understanding of the pathogenesis of vascular diseases and the development of new therapies, such as the prediction of cerebral aneurysm rupture and the selection of optimal therapeutic techniques, including more targeted arterial interventions.

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**Conflicts of Interest**

The authors declare that they have no conflicts of Interest.

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