

Published in final edited form as:

Physiol Rev. 2011 January ; 91(1): . doi:10.1152/physrev.00047.2009.

Effects of Disturbed Flow on Vascular Endothelium: Pathophysiological Basis and Clinical Perspectives

Jeng-Jiann Chiu and Shu Chien

Division of Medical Engineering Research, National Health Research Institutes, Taiwan; UST-UCSD International Center of Excellence for Bioengineering, National Yang-Ming University, Taiwan; and Departments of Bioengineering and Medicine and Institute of Engineering in Medicine, University of California, San Diego, La Jolla, California

Abstract

Vascular endothelial cells (ECs) are exposed to hemodynamic forces, which modulate EC functions and vascular biology/pathobiology in health and disease. The flow patterns and hemodynamic forces are not uniform in the vascular system. In straight parts of the arterial tree, blood flow is generally laminar and wall shear stress is high and directed; in branches and curvatures, blood flow is disturbed with nonuniform and irregular distribution of low wall shear stress. Sustained laminar flow with high shear stress upregulates expressions of EC genes and proteins that are protective against atherosclerosis, whereas disturbed flow with associated reciprocating, low shear stress generally upregulates the EC genes and proteins that promote atherogenesis. These findings have led to the concept that the disturbed flow pattern in branch points and curvatures causes the preferential localization of atherosclerotic lesions. Disturbed flow also results in postsurgical neointimal hyperplasia and contributes to pathophysiology of clinical conditions such as in-stent restenosis, vein bypass graft failure, and transplant vasculopathy, as well as aortic valve calcification. In the venous system, disturbed flow resulting from reflux, outflow obstruction, and/or stasis leads to venous inflammation and thrombosis, and hence the development of chronic venous diseases. Understanding of the effects of disturbed flow on ECs can provide mechanistic insights into the role of complex flow patterns in pathogenesis of vascular diseases and can help to elucidate the phenotypic and functional differences between quiescent (nonatherogenic/nonthrombogenic) and activated (atherogenic/thrombogenic) ECs. This review summarizes the current knowledge on the role of disturbed flow in EC physiology and pathophysiology, as well as its clinical implications. Such information can contribute to our understanding of the etiology of lesion development in vascular niches with disturbed flow and help to generate new approaches for therapeutic interventions.

I. INTRODUCTION

Vascular endothelial cells (ECs), which form the inner lining of blood vessel wall with direct exposure to blood flow, serve important homeostatic functions in response to various chemical and mechanical stimuli (87, 111, 125, 337, 485, 573). Besides providing a selective barrier for macromolecular permeability, ECs can influence vascular remodeling via the production of growth-promoting and -inhibiting substances; modulate hemostasis/thrombosis through the secretions of procoagulant, anticoagulant, and fibrinolytic agents;

Copyright © 2011 the American Physiological Society

Address for reprint requests and other correspondence: S. Chien, Dept. of Bioengineering, Univ. of California, San Diego, La Jolla, CA 92093-0427 (shuchien@ucsd.edu).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

mediate inflammatory responses via the surface expression of chemotactic and adhesion molecules and release of chemokines and cytokines; and regulate vascular smooth muscle cell (SMC) contraction through the release of vasodilators and vasoconstrictors (337). Endothelial dysfunction may lead to pathophysiological states that contribute to the development of vascular disorders resulting from atherosclerosis, thrombosis, and their complications (36, 93, 156, 203, 209, 493, 571).

While certain types of hemodynamic forces are essential for physiological functions of the EC under normal conditions, other types can induce endothelial dysfunction by adversely modulating EC signaling and gene expression, thus contributing to the development of vascular pathologies (42, 87, 93, 212, 213, 337, 360, 416, 451, 485, 571, 625), in concert with the risk factors that act on the entire arterial system (e.g., genetics, biochemical factors, living habits, etc.) (220, 253, 641). The possible role of hemodynamic forces in endothelial dysfunction was first suggested by the observation that the earliest lesions of atherosclerosis characteristically develop in a nonrandom pattern, i.e., preferentially at arterial branches and curvatures (or bends) (Fig. 1), where the local flow is disturbed. The disturbed flow pattern includes recirculation eddies and changes in direction with space (flow separation and reattachment) and time (reciprocating flow) (Fig. 2) (16, 39, 68, 69, 108, 111, 183, 186, 189, 190, 207, 210, 216, 217, 292, 311, 339, 393, 406, 416, 454, 492, 494, 502, 513, 534, 535, 545, 590, 595, 644, 645). Recent studies indicate that such disturbed flow and the associated low and reciprocating shear stress induce a sustained activation of a number of atherogenic genes in ECs, e.g., the monocyte chemotactic protein-1 (MCP-1) that induces monocyte infiltration into the arterial wall (86, 87, 256, 257, 265, 337, 520, 523–525) and platelet-derived growth factors (PDGFs) that enhance EC turnover and SMC migration into the subintimal space (309, 361, 616). In contrast, the straight part of the artery, which is generally spared from atherosclerotic lesions, is exposed to sustained laminar blood flow and high shear stress (with a definite direction), with the associated downregulation of atherogenic genes (e.g., MCP-1 and PDGF-BB) and upregulation of antioxidant and growth-arrest genes in ECs (37, 86, 87, 257, 266, 337, 361, 573). These findings suggest that disturbed and laminar flow patterns may induce differential molecular responses in ECs to result in, respectively, the preferential localization of atherosclerotic lesions at arterial branches and curvatures and the sparing of the straight parts of the arterial tree (135, 438). Disturbed flow may also occur in the aortic side of the valvular leaflets, which may regulate valvular endothelial signaling and phenotype that contribute to the preferential susceptibility to lesion development in this region (58, 129, 636). In the venous system, disturbed flow associated with reflux (i.e., retrograde flow) through dysfunctional or incompetent valves, outflow obstruction or stasis, or their combination, may cause venous hypertension, which may induce venous EC dysfunction and inflammation, and consequently result in the development and progression of chronic venous diseases, including varicose veins, deep venous thrombosis, and chronic venous insufficiency (36). Understanding of the effects of disturbed flow on EC signaling, gene expression, structure, and function will help to define the molecular and mechanical bases for the role of complex flow patterns in the development of vascular pathologies such as atherosclerosis, thrombosis, in-stent restenosis, bypass graft occlusion, transplant vasculopathy, aortic valve calcification, and venous valvular incompetence, as well as their clinical complications. Furthermore, such information may lead to the discovery and identification of new disease-related genes and the development of novel therapeutic strategies.

In this article, we provide an overview of the current experimental and theoretical knowledge on the effects of disturbed flow on ECs, in terms of signal transduction, gene expression, and cellular structure and function, as well as clinical relevance and applications. While our main objective is to review vascular endothelial responses to disturbed flow in vivo observed at branch points, curvatures, valve sinuses, and poststenotic regions, we also

discuss in vitro flow channel studies on the responses of cultured ECs to flow patterns without a clear direction versus pulsatile flow with a clear direction. Such in vitro studies allow a more detailed analysis of the molecular and cellular bases of mechanotransduction under these flow conditions that simulate what occur in different parts of the vasculature. We summarize our understanding of the pathophysiological mechanisms of lesion development in regions of disturbed flows that occur naturally in certain vascular niches or evolves following clinical interventions, and discuss their relevance to clinical application and perspectives.

II. HEMODYNAMIC FORCES AND ENDOTHELIAL DYSFUNCTION

This section provides an overview of the fundamental basis of hemodynamic forces acting on the vessel wall and their influences on vascular homeostasis and remodeling, as well as the pathophysiological factors contributing to endothelial dysfunction. These fundamentals help in the understanding of the mechanisms underlying the etiology of lesion development in vascular niches with disturbed flow, and hence the clinical occurrence of disturbed flow and its pathophysiological role to be discussed in section III.

A. Hemodynamic Forces, Vascular Homeostasis, and Remodeling of Blood Vessels

Blood vessels are constantly subjected to various types of hemodynamic forces (including hydrostatic pressure, cyclic stretch, and fluid shear stress) induced by the pulsatile blood pressure and flow (Fig. 3). As a monolayer in direct contact with the flowing blood, ECs bear most of the wall shear stress, which is the component of frictional forces arising from blood flow and acting parallel to the vessel luminal surface (125, 192, 337). The magnitude of shear stress in straight vessels can be estimated as being directly proportional to the viscosity of blood and inversely proportional to the third power of the inner radius of vessel (192, 360). Experimental measurements using different methods have shown that in humans the magnitude of shear stress ranges from 1 to 6 dyn/cm² in the venous system and from 10 to 70 dyn/cm² in arteries (85, 360, 417). The magnitude of shear stress in the normal mouse aorta is much higher (approximately an order of magnitude) than in humans (82, 552), consistent with the concept of allometric scaling that relates structure or function among species of vastly different sizes (224). In vivo observations indicate that increases or decreases in shear stress, rather than the absolute levels of shear stress, play critical roles in vascular homeostasis and remodeling (82, 281, 323, 465, 603). For example, increases in blood flow by constructing an arteriovenous shunt between the common carotid artery and the external jugular vein in dogs cause an initial increase in shear stress in shunted artery, which induces a compensatory expansion of the inner vessel radius of this artery within 6–8 mo to maintain the mean shear stress at the baseline level (281). Unilateral augmentation of femoral arterial flow induced by either acetylcholine administration or arteriovenous shunt elicits dilation with increase in diameter in 18 of 23 dogs, whereas the diameter of the contralateral control artery is not affected (465). Surgical manipulations by ligating the left common carotid artery of young rabbits at 3 wk of ages to increase laminar shear stress in the right carotid artery induce remodeling of this artery over 12 wk to increase the luminal diameter (or inner radius) (152), thus mitigating the increase in shear stress. A study of flow-induced arterial remodeling in rat mesenteric arteries suggests that this response involves the hyperplasia of both ECs and SMCs and an increase in synthesis of connective tissue in the vessel wall (580). The other side of the coin is that a decrease in shear stress resulting from the lowering of blood flow or blood viscosity can induce a decrease in inner radius of the vessel resulting from intima-media hyperplasia or wall hypertrophy (306, 307, 323, 375, 549). A 70% reduction in the rate of blood flow in the common carotid artery of rabbits caused a 21% decrease in the diameter of this artery within 2 wk (323). This decrease in arterial diameter induced by flow reduction is endothelium dependent and not relieved by

the vascular SMC relaxant papaverine, suggesting the occurrence of structural changes in the vessel wall rather than an increased vascular SMC contraction (323). Reduction in shear stress in a ligated unilateral common carotid artery in mice results in remodeling and formation of neointima hyperplasia containing BrdU-positive and SM -actin-positive cells (481). Such positive remodeling induced by increased shear stress (i.e., eutrophic vessel enlargement) and negative remodeling induced by decreased shear stress (i.e., hypertrophic vessel narrowing) are also important during normal embryonic and postnatal growth and use-dependent hypertrophy or atrophy of tissues (471). The signaling molecules involved in the flow-induced arterial remodeling include fibroblast growth factor-2 (549), adrenergic agents (170), nitric oxide (NO) (639), caveolin-1 (639), tissue transglutaminase [a calcium-dependent enzyme that induces protein cross-linking via α -(γ -glutamyl)lysine bonds] (20), G protein-coupled receptors (572), receptor tyrosine kinases (572), Axl (a receptor tyrosine kinase whose ligand is growth arrest-specific protein 6) (307), ATP-gated P2 \times 4 ion channel (632), heparin-binding epidermal growth factor-like growth factor (a potent vascular SMC mitogen) (572, 649), and cyclophilin A [a 20-kDa chaperone protein secreted from vascular SMCs in response to reactive oxygen species (ROS) to stimulate their proliferation and migration] (501). The compensatory arterial response to changes in shear stress during arterial remodeling requires an intact, functional endothelium and its ability to undergo adaptive adjustments in structure and function in response to shear stress alterations (206, 323, 464). The net effect of these endothelium-mediated compensatory responses is the maintenance of mean shear stress of the arterial system at \sim 15–20 dyn/cm² (208, 214, 360).

By connecting the external jugular vein to the common carotid artery in mice, Kwei et al. (315) and Abeles et al. (1) have demonstrated that the increases in shear stress and pressure in the vein following its exposure to arterial flow results in its arterialization within weeks, with adaptive responses of the vein wall, including structural and functional reorganization and changes in gene expression profiles. Expression of endothelial adhesion molecules E-selectin and vascular adhesion molecule-1 (VCAM-1) is observed at early time points, concomitant with the presence of neutrophils and monocytes/macrophages in the vascular wall. In addition, endothelium-dependent permeability is decreased in the arterialized vein compared with the contralateral control vein (315). When human saphenous vein segments are perfused *ex vivo* under arterial versus venous conditions, a significant increase in the production of metalloproteinases (MMPs) with cell proliferation under arterial flow is observed (196, 367). In vein bypass grafting, vascular SMCs migrate and proliferate in the intima, and the vessel wall changes in response to the new arterial environment and its associated shear stress, oxygen tension, metabolite concentrations, and pH (1, 123). Thus exposure to the new biomechanical environment of the arterial circulation is thought to be an important stimulus for the vascular remodeling of a venous bypass graft (73, 315).

In the venous system, changes in hemodynamic forces in the vein wall, including shear stress and pressure, may induce inflammation and the subsequent remodeling of the wall and venous valves, which are the fundamental mechanisms underlying various venous pathologies, including telangiectasias, reticular veins, and varicose veins (424, 446, 448). Study of surgical specimens and direct observation by angioscopy have revealed that the wall and valves of varicose veins undergo profound morphological changes, with thickening in some segments of the wall and thinning in others, dilation of the valve annulus, bulging and stretching of the valvular leaflets, and even complete destruction of the valves (448). These remodeling processes of the vein wall and venous valves involve the complex interplay of a range of factors, including an altered ratio between MMPs and their tissue inhibitors (TIMPs), and elevated levels of cytokines and growth factors that favor an alteration of the extracellular matrix (ECM) (424). In rodent models of venous thrombosis created by ligation of inferior vena cava to produce venous hypertension and altered shear stress (35), thrombus initiation is associated with a rapid vein wall inflammatory reaction

involving early endothelial activation and neutrophil infiltration (35, 157), and later vein wall remodeling associated with increased expressions of MMP-9 and MMP-2 (35, 141). These results on the venous system, in concert with the observations on the arterial system, suggest that changes in hemodynamic forces play critical roles in the remodeling of blood vessels, including both arteries and veins.

B. Endothelial Dysfunction Is a Critical Pathophysiological Factor in Vascular Diseases

The involvement of vascular endothelium in disease processes such as atherosclerosis and thrombosis has been recognized since the time of Virchow (591), but the mechanistic insight into the pathobiology of this process has been developed only recently. It is now appreciated that the normal function of the single-cell-thick endothelial lining of the circulatory system is essential for vascular homeostasis in health and that its dysfunction plays a significant role in many vascular diseases. Clinical evaluation of endothelial function in humans has focused primarily on endothelium-dependent changes in vascular SMC contractility, which is mediated by the release of vasodilators [i.e., endothelium-derived relaxing factors such as NO and prostacyclin (PGI₂)] and vasoconstrictors [i.e., endothelium-derived contracting factors such as thromboxane A₂, free radicals/ROS, endothelin (ET), and angiotensin II] (159, 174, 175, 236). Endothelial dysfunction is often associated with a paradoxical vasoconstriction in response to vasodilating agents such as acetylcholine, due to an impaired activity of endothelial NO synthase (eNOS) or the inactivation of NO by free radicals/ROS such as superoxide anion (236). Paradoxical vasoconstriction with acetylcholine occurs because acetylcholine interacting with its receptors on SMCs leads to vasoconstriction unless inhibited by the acetylcholine-NO axis (159, 352). Several studies have demonstrated that the early stages of atherosclerosis are characterized by a decreased NO bioavailability as a possible consequence of inactivation by superoxide anion (17, 384, 605). In addition to impairments of endothelium-dependent vasodilation, other pathophysiological consequences of endothelial dysfunction specific to atherosclerosis include the following (43, 213, 330):

1. Abnormal vascular reactivity and vasospasm that may cause ischemia, angina, and myocardial infarction;
2. Increased permeability to macromolecules such as lipoproteins;
3. Increased expression of chemotactic molecules (e.g., MCP-1) and adhesion molecules [e.g., intercellular adhesion molecule-1 (ICAM-1), VCAM-1, and E-selectin/P-selectin];
4. Enhanced recruitment and accumulation of monocytes/macrophages in the intima as foam cells;
5. Altered regulation in growth and survival of vascular cells, e.g., decreased EC regeneration and increased SMC proliferation and migration;
6. Disturbed hemostatic balance of thrombotic and fibrinolytic states, e.g., increased expression of procoagulatory molecules such as von Willebrand factor (vWF) and tissue factor (TF), enhanced thrombin generation, platelet aggregation and adhesion, and fibrin deposition.

The pathophysiological factors leading to endothelial dysfunction that are especially relevant to the development of vascular disease include the following (2, 43, 213, 330, 338, 492, 493):

1. Activation by cytokines;
2. Stimulation by free radicals/ROS or oxidative stresses and the advanced glycation end products generated in diabetes and aging;

3. Chronic smoking and hypertension;
4. Chronic exposure to hyperhomocysteinemia and/or hypercholesterolemia;
5. Increased plasma levels of oxidized low-density lipoprotein (LDL) and its accumulation within the vessel wall, as well as infection by bacteria, viruses, and other pathogens with the release of their products (251, 435, 528, 618).

There is increasing evidence that various hemodynamic forces, including fluid shear stress, can also influence the structure and function of ECs and modulate their expressions of pathophysiologically relevant genes (74, 87, 93, 125, 131, 153, 203, 211, 213, 230, 240, 279, 282, 337, 571, 625). The pulsatile flow in the straight part of the arterial tree is laminar with high shear stresses (10–70 dyn/cm²) (208, 214, 360). Vascular ECs subjected to laminar flow with high shear stress constantly release NO (56, 431, 582). The vascular geometries at arterial branch points, curvatures, and post-stenotic regions lead to disturbances in flow pattern, with a low net forward flow and shear stress (<4 dyn/cm²), such complex flow patterns without a clear direction play significant roles in inducing endothelial dysfunction, with impairment of NO production (93, 103, 213, 360, 545, 571). At sites of lesion predilection, disturbed blood flow coupled with hyperlipidemia results in EC activation, which leads to fatty streak formation (541). In humans, endothelium-dependent vasodilation is impaired at the branches of coronary arteries (103, 373). An increase in the backward component of pulsatile flow by inflating an occluding cuff around one forearm of healthy men (24 ± 3 yr) to 25, 50, or 75 mmHg for 30 min induces a dose-dependent attenuation of flow-mediated vasodilation in the brachial artery of this arm (565). In the uncuffed arm, no changes in shear rate or flow-mediated dilation are observed (565). In the aorta of hypercholesterolemic LDL receptor-deficient (LDLR^{-/-}) mice, the down-regulation of eNOS expression and the upregulation of proatherogenic transcription factors (i.e., Elk-1 and p-CREB) (103, 140) are particularly pronounced at the lesion-prone sites with disturbed flow. An *ex vivo* study using porcine left common carotid segments has shown that low and reciprocating shear stress (0.3 ± 3 dyn/cm²) decreases the expression of eNOS gene and NO-mediated vasodilation in response to bradykinin, compared with the unidirectional laminar shear stress (6 ± 3 dyn/cm²), suggesting that plaque-prone hemodynamic flow patterns impair endothelial function in carotid arteries (199). The endothelial dysfunction (with reductions of eNOS expression and NO production) induced by decreases in blood flow and shear stress can be reversed by physical training to increase the level of shear stress (160, 237, 238). The current state of research on the effects of hemodynamic forces on endothelial biology and pathobiology indicates that elucidation of the role of disturbed flow pattern in endothelial function and dysfunction can provide insights into the mechanisms that predispose specific regions in the vascular system to pathophysiological processes and may help to develop hemodynamic-based strategies for the prevention and management of vascular diseases resulting from atherosclerosis and thrombosis.

III. OCCURRENCE OF DISTURBED FLOW IN PATHOPHYSIOLOGICAL CONDITIONS

Disturbed flow occurs in the vascular system in a number of conditions, including 1) flow disturbance that naturally occurs in certain regions such as branch points and curvatures of large arteries and the aortic and venous valve sinuses; 2) flow disturbance generated in arteries through surgical interventions such as end-to-side anastomosis in bypass graft and stent insertion following balloon angioplasty; 3) flow disturbance in the venous system due to reflux through incompetent valves or outflow obstruction; and 4) temporal changes in local levels of flow due to sudden cessation and reperfusion in clinical procedures such as organ transplantation. These disturbed flow conditions contribute to the pathogenesis of

many clinical disorders, including atherosclerosis, arterial aneurysm, aortic valve calcification, postsurgical intimal hyperplasia, in-stent restenosis, venous varicosity, chronic venous insufficiency, ischemia/reperfusion injury, and transplant vasculopathy.

A. Correlation of Locations of Atherosclerotic Lesions With Regions of Disturbed Flow in the Arterial Tree

The correlation between the locations of atherosclerotic lesion and the regions of disturbed flow is well documented throughout the arterial system (16, 68, 207, 215–217, 298, 311, 393, 454, 455, 494, 502, 534, 535, 552, 563, 590, 595, 644). These include carotid bifurcations (65, 217, 311, 644, 645), aortic arch (68, 215, 292, 563, 571) (Fig. 4) and branch points of coronary (16, 188, 207, 215, 391, 494, 544), infrarenal, and femoral arteries (393, 454, 455, 534, 535, 563, 595, 613) (Table 1). The nonrandom distribution of atherosclerotic lesions holds true not only for various experimentally induced atherosclerosis (dietary and/or genetic models) and across multiple animal species (monkeys, rabbits, pigs, and rodents), but also for the natural history of this pathological process in humans (31, 55, 68, 105, 106, 109, 205, 215, 406, 502, 547, 563, 590, 623, 644, 651). This site selectivity of atherosclerotic lesions is attributable, at least in part, to the modification of local phenotype and function of vascular ECs by the disturbed flow pattern (viz. flow separation, recirculation, reattachment), low and reciprocating shear stress, and high spatial and temporal gradients of shear stress (263) (Fig. 5). The local differences in flow patterns (laminar vs. disturbed) and in shear stress magnitude or gradient may explain the different rates of atherosclerosis progression among different vessels within the same individual (and even different segments within the same vessel), despite their exposure to the same systemic risk factors such as lipoprotein concentrations (207, 622). Advanced lesions in arteries may cause the weakening of the artery wall, leading to pressure-induced aneurysm and potential rupture, which is also mediated by the complex flow conditions within the aneurysm (476, 498, 514).

B. Contribution of Complex Flow Environments to the Focal Nature of Calcific Lesions in the Aortic Valve

Disturbed flow may occur in the aortic valve sinus and serve as a major contributing factor for valvular heart disease (129, 636). In vitro or ex vivo modeling of aortic valve dynamics suggests that the two sides of the aortic valvular leaflets are exposed to different types of shear stress during systole: low and reciprocating shear stress without a clear direction on the aortic side and unidirectional pulsatile shear stress on the ventricular side (58, 122, 425, 495). This side specificity is in concert with the correlation found between the preferential locations of calcific lesions on the aortic side of the valve and the local hemodynamics experienced by the valvular leaflets (60). In humans (432, 441) and pigs (529), lipid deposits and calcific lesions in the aortic valve occur preferentially in the fibrosa (the layer of the valve immediately beneath the endothelium) on the aortic side of the valve, where complex flow patterns are likely to occur. The preferential susceptibility to lesion formation on the aortic rather than ventricular side of the aortic valve may result from differential regulation of EC gene expression on the two sides, leading to side-specific endothelial phenotypes that favor or inhibit calcification, respectively (129). Thus there is a spatial correlation between the focal nature of calcific lesions and the local hemodynamic environment, similar to that observed for focal atherosusceptibility in the arterial tree (58, 129).

C. Flow Disturbance Associated With Vascular Surgical Interventions

Several types of surgical interventions have been developed with the aim of restoring blood flow in vascular occlusive diseases. These interventions, however, may themselves induce regions of disturbed flow or low shear conditions, thus leading to neointimal hyperplasia and atherosclerotic lesions with poor blood flow that negatively influence the long-term efficacy

of the treatments (472, 568). These clinical conditions include restenosis following angioplasty, in-stent restenosis, and bypass graft failure.

Balloon angioplasty is one of the most common modalities of treatment of coronary artery narrowing and occlusion, but a significant percentage of patients undergoing percutaneous balloon angioplasty would develop restenosis, with vessel wall thickening recurring within the first 6 mo in 30–40% of these cases (289, 394, 420). The frequency of restenosis after angioplasty has been correlated with impaired blood flow with low shear stress, which augments the inward vessel remodeling, cell migration, and activity of genes regulating migration, and significantly exacerbates the reduction in luminal size observed several months after the procedure (511, 604).

The use of stents in angioplasty has dramatically increased since their approval by the United States Food and Drug Administration in 1994, and there have been significant and technological advances. However, restenosis affects 20–40% of de novo coronary lesions treated with traditional bare metal stents (164, 167, 287, 288, 569). This in-stent restenosis is believed to be influenced by abnormal wall stress and disturbed blood flow around the stent (162, 298, 499). Intravascular ultrasound has shown that stent placement in diseased arteries results in a “step-up” phenomenon at the proximal edge of the stent, with the existence of regions with retrograde velocities and flow separation (568). These regions are prone to neointimal hyperplasia and hence in-stent restenosis, suggesting the role of disturbed flow with low and reciprocating shear stress in the development of in-stent restenosis. A randomized, double-blind trial comparing a sirolimus-eluting stent with a bare metal stent in 1,058 patients at 53 centers in the United States who had a newly diagnosed lesion in a native coronary artery has demonstrated that the rate of failure of the target vessel (a composite of death from cardiac causes, myocardial infarction, and repeated percutaneous or surgical revascularization of the target vessel) within 270 days was reduced from 21% with a bare metal stent to 8.6% with a sirolimus-eluting stent (394). Although the drug-eluting stent has led to a reduction in restenosis rate, the optimal approach to treat obstructive atherosclerotic lesions in arterial bifurcations remains unresolved, probably due to the complex patterns of flow and plaque distribution and vessel geometry in these regions (489). It has been estimated that coronary bifurcation lesions are involved in up to 10% of all percutaneous coronary interventions (33). Recent studies on autopsy series have shown a greater prevalence of late stent thrombosis in bifurcation lesions treated with a drug-eluting stent compared with a bare metal stent (176, 409). This late stent thrombosis induced by drug-eluting stent is accompanied by an impaired arterial healing at the flow divider, where the flow disturbance is increased after stent placement, compared with the lateral wall sites (409). Thus the long-term outcomes of the use of drug-eluting stents in bifurcation lesions may be tempered by an increased risk for thrombosis by stent-induced flow disturbances, which may influence drug deposition, arterial healing poststenting, and local fibrin and platelet deposition (21, 177, 267, 304, 408, 409).

Neointima hyperplasia is a major cause of late graft failure in arterial bypass grafting using either artificial vascular graft or a vessel segment from the patient, usually the saphenous vein (102). The insertion of a segment of the saphenous vein into the arterial system exposes the thin-walled and compliant vein to the pressure and shear stress in the arterial system that are much higher than what the venous wall is prepared to accommodate. This mechanical mismatch may cause the saphenous vein segment to bulge, thus resulting in a geometric mismatch that would lead to disturbed flow and vulnerability to neointimal hyperplasia and atherosclerosis downstream of the anastomosis, with the subsequent failure of bypass grafts (86, 295, 348). In addition, the saphenous vein is generally larger in diameter than the grafted coronary artery, resulting in reduced linear velocity of flow in grafting (194, 531). Any existing bicuspid valves in the saphenous vein graft may cause further impediment and

become sites of flow disturbance, especially when two valves are present in the body of a single saphenous vein graft (530). Thrombus formation at or distal to these valves results in early graft occlusion after bypass surgery (382, 530, 533). Surgical modifications using vein cuffs to shape the curvatures of distal anastomosis of arterial bypass grafts can alter the location of low shear stress, with a corresponding shift in the area of neointimal hyperplasia (297), indicating that disturbed flow with low shear stress plays important roles in mediating the process of neointimal hyperplasia. Using the photochromic tracer technique to visualize the variations of shear stress in a 30° anastomosis in the absence (disease-free model) or presence of intimal hyperplasia (disease model produced by fitting the wall of the native artery with an elliptical plug to simulate the presence of intimal hyperplasia on the bed), Ojha (434) showed the occurrence of reduced gradients of shear stress downstream to the anastomosis in the diseased model compared with the disease-free model, suggesting that the development of anastomotic intimal hyperplasia may initially be a compensatory response resulting in a reduction of disturbed flow region.

D. Disturbed Flow Pattern Around Venous Valves and Its Pathophysiological Role For Thrombosis Initiation

Veins bring partially deoxygenated blood from the organs and tissues to the right heart and lungs, where it is reoxygenated. The venous system of the lower limbs consists of an interconnected network of superficial veins (e.g., great and lesser saphenous veins), perforator veins, and deep veins (e.g., femoral and popliteal veins) (478). A series of bicuspid valves is located throughout the superficial and deep veins to ensure blood flow movement in the cephalad direction, preventing the reflux of blood toward the feet while in the upright posture (163, 396). Perforating veins also contain one-way valves that prevent reflux of blood from the deep veins into the superficial system. Early studies on the flow behaviors of model particles and red blood cells through an isolated transparent dog saphenous vein containing a two-leaflet valve show a large primary recirculation eddy as well as a small secondary eddy in each valve pocket (285, 286). Developments in ultrasound technology supplemented by B-mode and pulsed-wave Doppler scanning have allowed detailed noninvasive *in situ* visualization of the valve with its cusps and the flow of blood through the valve (354). In normal physiological conditions, venous flow is pulsatile and venous valves open and close ~20 times/min while a person is standing (8, 36, 354). The movements of the valve cusps undergo four phases that constitute the valve cycle. In the opening phase, the cusps move from the closed position toward the sinus wall. In the equilibrium phase, the valves cease opening and the leading edges remain suspended in the flowing stream and undergo self-excited oscillations that resemble flutter of flags in wind. In the closing phase, the leaflets move synchronously toward the center of the vein. In the closed phase, the cusps remain closed (354). During the equilibrium phase, flow through the valve separates into a proximally directed jet in the center of vein and a recirculation eddy into the sinus pocket behind the valve cusp with reattachment at the wall of the sinus (Fig. 6). The central jet possibly facilitates outflow, and the recirculation eddy prevents stasis in the pocket to ensure all surfaces of the valve being exposed to shear stress. Valve closure occurs when the pressure caused by the recirculation eddy exceeds the pressure on the luminal side of the valvular leaflets as a result of the proximally directed jet. Thus venous valves are operated by pressure rather than driven by flow so that little or no reflux occurs in bringing about complete closure of the valve (36, 475).

There are several features of the venous valves that render these regions highly vulnerable to the initiation and formation of thrombosis (8). First, ECs lining the valve pocket are exposed to disturbed flow with reduced shear stress or even stasis due to immobilization during all phases of the valve cycle (354). This reduced flow or stasis in the valve sinus is shown by the finding that contrast media linger in valve sinuses for an average of 27 min post-

venography (372). This valve sinus stasis may modulate the expression of thrombotic factors in these ECs and prevent the efflux of activated clotting factors and influx of clotting factor inhibitors, constituting a potentially hypercoagulable microenvironment (52). Second, the blood in the cusps of the valve is relatively hypoxic (235), which has been well documented to induce inflammatory responses of ECs, with increased generation of intracellular ROS that plays important roles in vascular pathologies (238, 366). Third, there is a tendency for white blood cells (WBCs) and platelets to become trapped in the valve pocket due to flow recirculation or stasis. For example, Ono et al. (436) have shown infiltration of valvular leaflets and the venous wall by WBCs (monocytes and macrophages) in all valve specimens from patients with chronic venous diseases, but in none from controls. Further work by Takase and co-workers (559, 561) has confirmed this phenomenon. This is in keeping with Virchow's triad (49, 262, 276, 316, 359, 606), which indicates that local thrombosis may occur when the flow velocity or pulsatility is reduced, the hemostatic balance is altered, and/or the vascular integrity is lost (8). Indeed, direct evidence from autopsy studies and phlebography, as well as circumstantial evidence such as the correlation between the frequency of deep venous thrombosis and the number of valves in individuals, have revealed that the sinus of venous valves is a frequent location of thrombosis initiation (52, 452, 512). The thrombus begins as a microscopic nidus of fibrinred cell collections near the apex of the valve pocket (8). Regions of endothelium adjacent to venous valves are common sites of WBC and platelet accumulation and EC injury (169). Thus the sinus of venous valve is a unique microenvironment in some respects similar to the microenvironments present in other specific vascular niches prone to atherosclerosis and thrombosis.

E. Flow Disturbance Due to Blood Reflux Through the Incompetent Valves and Outflow Obstruction in the Venous System

Incompetence of venous valves, which may occur as a result of pathological dilatation, inflammation, or deterioration of the vein wall, clot-related scarring, or an inherited valve abnormality, allows venous reflux or retrograde flow, which leads to altered pressure and shear stress that underlie the clinical manifestations of chronic venous diseases, including varicose veins and chronic venous insufficiency (36, 222, 376, 478). A small degree of reflux may occur in the presence of a normally functioning valve (500). The duration of reflux (or the reflux time) of >0.5 (or 1.0) second is used to diagnose the presence of abnormal reflux, although a more refined definition with a variable "cutoff" value based on the location of the vein has been suggested (163, 318). A longer reflux time implies more severe disease. Other parameters such as the peak reflux velocity (>10 cm/s) and the calculated reflux volume have also been used to assess the severity of reflux (163, 319, 376, 412, 423).

The obstruction of the deep veins, which limits the return of blood from the limbs to the heart and results in venous hypertension (30, 415, 477), is also a major cause leading to chronic venous insufficiency and increasing the risk for pulmonary embolism. The most common cause of obstruction is deep vein thrombosis; other causes are inherited abnormalities, inadequate recanalization or venous stenosis, and compression of the vein, e.g., from a tumor or bandage (30). These clinical conditions may further damage and destroy venous valves, leading to the worsening of venous reflux. In patients with severe clinical diseases, combination of reflux and obstruction is seen more frequently than reflux or obstruction alone (415).

Mellor et al. (376) have demonstrated that mutations in the *Foxc2* gene, which is located on chromosome 16 (421) and is expressed in both ECs and SMCs of developing blood vessels (313) and on the venous and lymphatic valvular leaflets (457, 621), are strongly associated with primary valve failure in veins of the lower limb and result in lymphedema distichiasis, an inherited primary lymphedema in which a significant number of patients have varicose

veins (376). Investigations by color Duplex ultrasound in 18 participants with a Foxc2 mutation reveal reflux in the great saphenous vein that every one of the 18 (compared with only 1 of 12 referents that include 10 family members), and that 14 of these 18 participants also have deep vein reflux (376). The extent of disturbance in venous flow by reflux depends on the magnitude of retrograde flow, which is generally greater in refluxing superficial veins than in refluxing deep veins (376). The walls and valves of the superficial veins are more prone to structural failure than the deep veins, probably because the deep veins are better supported by muscle and fascia, whereas superficial veins are only surrounded by loose connective tissue and fat (163, 376). This is in keeping with the finding that superficial veins fail before deep veins during the progression of venous disease (317, 376, 390). Chronic venous reflux is associated with increases in interactions between WBCs and ECs (34) and the numbers of activated WBCs (436), which can lead to the activation of ECs and upregulation of proinflammatory genes that underlie the clinical manifestations of chronic venous diseases (34).

F. Flow Disturbance Due to Stagnation and Reperfusion

An additional form of flow disturbance that mediates endothelial dysfunction is the cessation or stagnation of flow and its later recovery in clinical conditions associated with ischemia/reperfusion or hypoxia/reoxygenation injury, which complicates myocardial recovery following acute infarction, and can result in cell damage, arrhythmia, and death (182, 503, 612). Endothelial dysfunction is also associated with tissue injuries induced by trauma resuscitation, solid organ transplantation, and major vascular surgical interventions, which result in inflammatory responses with recruitment of WBCs (such as neutrophils) from the microcirculation (4, 460, 496). Hypoxia and reoxygenation have been well documented to induce EC inflammation and EC-WBC interactions, which play important roles in the pathogenesis of vascular diseases (305, 366). For more information about vascular/endothelial responses to ischemia/reperfusion or hypoxia/reoxygenation injury, the reader is referred to several excellent reviews (4, 66, 223, 299, 325, 597).

IV. EFFECTS OF DISTURBED FLOW ON VASCULAR ENDOTHELIUM IN VITRO

A. In Vitro Models For Studying the Effects of Disturbed Flow on ECs

In the past decades a variety of in vitro systems for applying shear flow to ECs have been developed in an attempt to reproduce important features of in vivo flow environments of ECs to gain insight into the mechanisms of their responses to shear stress. These systems include those based on the principles of parallel-plate flow chamber (185, 198, 245, 308, 326, 333), cone-and-plate viscometer (40, 132, 149, 150, 249, 504), parallel disk viscometer (324), orbital shaker (453, 640), and rectangular or tubular capillary tube (392, 456, 482) (Fig. 7). The systems using parallel disk viscometer and orbital shaker do not result in a uniform shear stress across the entire monolayer, and the capillary tubes do not yield sufficient amounts of cells for some bioassay analysis. Systems based on the principles of parallel-plate flow chamber and cone-and-plate viscometer have been widely used for studying EC responses to shear stress in terms of molecular signaling and gene expression, because of their capability of producing a wide range of magnitudes of uniform shear stress on ECs and thus allowing the collection of sufficient amount of cells for analysis. The parallel-plate flow channel is created by using a gasket with a rectangular cutout to result in a uniform channel height along the path of flow, which is commonly generated between two slit (manifold) openings at either end of the rectangular chamber by a constant pressure head, syringe pump, or peristaltic pump (Fig. 7A). This parallel-plate approach holds many practical attractions, including homogeneity of the force stimulus, simplicity of the equipment, ease of medium sampling/exchange, and ease of access to the culture (both

physically and for microscope visualization) (53). The flow in the cone-and-plate system is generated by rotation imposed around a cone axis oriented perpendicular to the surface of a flat plate (Fig. 7B). Since both the local relative velocity and the separation between the cone and plate surfaces vary linearly with the radial position, this configuration achieves spatially homogeneous shear stress on both surfaces, as well as the fluid in the gap (53). Depending on the cone taper and the imposed angular velocity, a wide range of shear stresses can be achieved, extending even into the turbulent flow regime (53, 132). Direct microscopic visualization is also feasible (504), albeit somewhat cumbersome, with the advantage of small volumetric fluid requirement (53). All these devices allow the determination of the effects of uniform laminar shear stress on cultured endothelial cells. To study the effect of spatial variation of laminar shear stress on ECs, Usami et al. (584) designed a parallel-plate flow chamber in which a tapered channel is used to create a linear variation of shear stress, starting from a predetermined maximum value at the entrance and falling to zero at the exit, enabling the study of EC responses to a wide range of shear stress using a single run at constant flow.

Several in vitro models capable of producing disturbed flow have been established on the basis of parallel-plate flow chamber and cone-and-plate viscometer to simulate some of the features of complex flow patterns in the vascular tree. In the cone-and-plate viscometer, this can be achieved by imposing a rectangular obstacle. In the parallel-plate flow chamber, a step near the inlet can be created by using two silicone gaskets with the top one having a longer longitudinal cutout than the one below (Fig. 8), resulting in a backward-facing step (i.e., vertical-step) flow in the channel (28, 76, 88, 94, 134, 147, 148, 259, 349, 379, 405, 458, 532, 564, 576). The step-flow channel is characterized by a well-defined recirculation eddy immediately downstream of the step, followed by a region of flow reattachment and finally a unidirectional laminar flow that is reestablished further downstream (Fig. 9) (88, 94). When ECs are subjected to shear flow in a step-flow channel, their responses to both laminar and disturbed flows can be studied in the same monolayer (for summary, see Table 2). However, it is difficult to use standard immunoblotting techniques to analyze the effects of disturbed flow on EC signaling and gene expression in the step flow channel, because the local hemodynamic environments in the channel vary with a length scale of only tens to hundreds of micrometers with only a few cells being exposed to the flow disturbances (approximately hundreds of cells in a 1-mm span of the confluent monolayer) (134). Therefore, alternative experiments have been performed to study the effects of reciprocating flow in a parallel-plate flow chamber (with the use of a reciprocating flow pump) (87, 259), a cone-and-plate viscometer (266, 536, 537), or an orbital model (119). The aim of these reciprocating flow devices is to create a reciprocating flow without a significant forward direction, which is a main feature of the disturbed flow, that allows the acquisition of sufficient amount of cells for analysis by a variety of bioassays. These devices, however, do not allow the study of the responses of ECs to different shear flow patterns in the same chamber.

In an attempt to better approximate the actual wall shear stress experienced by ECs in arterial systems in vivo and to understand how these stimuli affect endothelial responses, Blackman et al. (41) have developed a cone-and-plate dynamic flow system that can produce pulsatile shear stresses in an arterial-like waveform that mimics the flow characteristics in human arteries, e.g., a previously defined waveform of the human abdominal aorta of a healthy volunteer that has been characterized by both magnetic resonance and ultrasound imaging techniques (41, 358). A series of research studies have been conducted using this sophisticated in vitro dynamic flow system with the impositions of actual shear stress waveforms from carotid bifurcations or coronary collateral vessels (115, 116, 355, 444), which represents a significant advance over the earlier studies on the responses of ECs to disturbed flow using a relatively simplified shear flow system. This

dynamic flow system is also used to investigate the differential role of arterial and venous shear stress waveforms (derived from human abdominal aorta and saphenous vein, respectively) in modulating the responses of arterial and venous ECs to cytokine tumor necrosis factor- α (TNF- α) (347). Research using these common in vitro shear flow systems has helped the investigation on EC functions in both physiological and patho-physiological conditions.

B. Effects of Disturbed Flow on EC Morphology, Cytoskeletal Organization, Proliferation, and Migration

ECs exposed to disturbed flow with a low and reciprocating shear stress (e.g., with a mean value of only ~ 0.5 dyn/cm² and a pulsatile oscillation of ± 4 dyn/cm²) and laminar flow with a relatively high shear stress (10–20 dyn/cm²) show differential responses, in terms of molecular signaling, gene expression, structure, and function. For example, ECs exposed to disturbed flow in a step-flow channel (i.e., areas *a* and *c*, with mean shear stress values of 0.5 and 0 dyn/cm², respectively) are round in shape (Fig. 10), with random and short actin filaments located mainly at the periphery of the cells (Fig. 11) (94). In contrast, ECs exposed to laminar flow (i.e., areas *b* and *d*, with opposite flow directions) with a relatively high shear stress (20 dyn/cm²) are aligned in the direction of flow, with the formation of very long, well-organized, parallel actin stress fibers in the central region (Figs. 10 and 11) (94, 147, 149, 172, 187, 243, 294, 333, 483). These results on EC morphological changes are comparable with in vivo observations in the primate thoracic aorta (107, 125, 128, 134) that ECs are elongated and aligned with the longitudinal axis of the vessel in the straight, nonbranch areas and that they have a polygonal morphology in regions around branches, where the shear stress oscillates back and forth over the cardiac cycle (Fig. 12). In vivo studies have also shown that low shear stress favors prominent peripheral actin microfilaments (294, 592), while high shear stress promotes an increase in central actin stress fibers (294, 322).

ECs exposed to disturbed flow in a step-flow channel turn over more rapidly than those kept in static conditions (86, 94, 148, 337, 564), with their DNA synthesis rate being significantly higher in the reattachment area than in the laminar flow area or under static condition (86, 87, 94) (Fig. 13). This enhancement of EC proliferation by disturbed flow is accompanied by a sustained activation of extracellular signal-regulated kinase (ERK) (337) and is suggested to be regulated by the inhibition of the p21^{CIP1}-suppression of cyclin-dependent kinase activity to allow G₀/G₁-S transition (9, 126). Turbulent fluid shear stress also induces vascular EC turnover in vitro (132). In contrast, a high level of laminar shear stress inhibits DNA synthesis (9, 94) and proliferation of ECs (334), with a reduction of the number of cells entering the cell cycle and arrest of the majority of cells in the G₀/G₁ phase (9, 342, 648). These in vitro results are comparable with the in vivo findings that ECs at the branch points of human iliac arteries (where disturbed flow predominates) have shorter telomeres and a focal acceleration of senescence (70, 103). Early studies on in vivo uptake of tritiated thymidine with subsequent autoradiography of Häutchen (en face) preparations of guinea pig aortic endothelium show that mitosis is most frequent in the aortic arch and around the orifices of branches, where the local flow is disturbed (626). Later studies on light microscopic examination of the luminal surface of the entire rabbit thoracic aorta also reveals that ECs in regions of disturbed flow exhibit higher mitotic rates (86, 97). Guo et al. (228) have shown that distinct modes of interactions between AMP-activated protein kinase (AMPK) and Akt play important roles in the regulation of EC cell cycle by laminar versus disturbed flows. Laminar shear stress activates both AMPK and Akt in ECs; the counterbalance between the antimitotic AMPK and the proliferative Akt results in relatively undisturbed mTOR (mammalian target of rapamycin) and its target p70 ribosomal S6 kinase (p70S6K). Thus EC entrance into the mitotic S and G₂/M phases is attenuated, and

endothelial homeostasis is maintained. In contrast, disturbed flow activates only Akt; the lack of AMPK activation to generate an antagonistic effect leads to a sustained activation of p70S6K and, consequently, a high proportion of ECs in the mitotic state.

Laminar shear stress significantly enhances EC migration in wound healing in the flow channel *in vitro* (11, 13, 259) and canine carotid-femoral grafts *in vivo* (628), whereas disturbed flow has no significant promoting effect on wound healing (Fig. 14). There is evidence that disturbed flow causes cell loss and the migration of ECs away from areas with a high shear stress gradient (564). The shear-induced directional migration is related to the shear-mediated lamellipodia protrusion and focal adhesions remodeling, which are regulated by Rho family small GTPases (133, 260, 336, 337).

C. Effects of Disturbed Flow on EC Permeability and Junctional Proteins

The intact intercellular junctions of the normal endothelium in the straight part of the arterial tree do not allow the passage of macromolecules such as lipoproteins, except during EC turnover (86, 345, 607). The atheroprone areas such as the branch points, where blood flow is disturbed, have an increase in permeability to macromolecules, including ^{131}I -albumin (32) and Evans blue dyelabeled albumin in the pig (250) and rabbit (86, 97) aorta, and low-density lipoprotein (LDL) or fluorescent, radiolabeled and chromogenic tracer particles in normal or cholesterol-fed rabbit aorta (506, 507, 540, 542, 611). Many of these *in vivo* observations have been reproduced in *in vitro* studies. A step-flow channel has been used to show that the transendothelial transport of dextran (mol wt 70,000) in the vicinity of flow reattachment is significantly higher than that in the laminar flow area (458). ECs in regions of disturbed flow in the rabbit thoracic aorta *in vivo* (128) and the step-flow channel *in vitro* (94, 148) also have higher mitotic or turnover rates compared with ECs in other regions, where flow is unidirectional, providing evidence in support of the “cell turnover-leaky junction hypothesis” (79, 97, 261, 344, 345, 609).

The modulations of the expression and structure of permeability-related intercellular junctional proteins, including connexins (Cx) and vascular endothelial (VE)-cadherin (104, 142, 147, 379) by disturbed flow provide insights into the mechanisms by which disturbed flow enhances endothelial permeability. Disturbed flow causes an upregulation of Cx43 in ECs, but the distribution of Cx43 is discontinuous at the EC periphery, as demonstrated by the *in vivo* observations at vascular branch points in the rat aorta (195) and in the intima of atherosclerotic lesions in humans and rabbits (466, 467), as well as *in vitro* experiments using a step flow channel (134, 147). *In vivo* studies on the rat aorta have shown that compared with the strong staining of VE-cadherin at EC borders in the descending thoracic aorta, where the pulsatile flow has a net forward component, there is only a faint staining in the aortic arch and the poststenotic dilatation site beyond an experimental constriction, where the flow is reciprocating with low net flow (379). *In vitro* flow channel experiments have shown that short-term exposure (6 h) of ECs to either reciprocating ($0.5 \pm 4 \text{ dyn/cm}^2$ at 1 Hz) or pulsatile ($12 \pm 4 \text{ dyn/cm}^2$ at 1 Hz) flow causes disruption of VE-cadherin and its associated protein β -catenin at cell borders (Fig. 15) (379). However, these junctional VE-cadherin and β -catenin molecules reappear as continuous distributions after sustained exposure (24 h or longer) to pulsatile flow, but not reciprocating flow (Fig. 15). The differential expressions and distributions of gap junction proteins in regions of disturbed flow may contribute to the junction discontinuity and consequently enhanced macromolecular permeability.

D. Effects of Disturbed Flow on EC Signaling and Gene Expression

By using microdissection and antisense RNA amplification methods, Davies et al. (134) demonstrate by DNA microarray studies that ECs isolated from disturbed flow regions

exhibit a greater degree of heterogeneity in gene expression than those from laminar flow regions and that the heterogeneity in disturbed flow regions may contribute to the focal initiation of atherosclerotic lesions (27, 130, 134). ECs subjected to disturbed flow in a step-flow channel exhibit localized increases in the levels of transcription factors such as nuclear factor- κ B (NF- κ B), early growth factor-1 (Egr-1), c-*fos*, and c-Jun in their nuclei (405) and enhanced expressions of ICAM-1 and E-selectin, but not VCAM-1, on their surfaces (88). ECs in the disturbed flow region of a step-flow channel also exhibit a sustained activation of sterol regulatory element (SRE) binding protein (SREBP), which acts to increase the SRE-mediated transcriptional activation of EC genes encoding for LDL receptor, cholesterol synthase, and fatty acid synthase, with the consequent enhancement of LDL uptake and lipid synthesis (349), compared with ECs exposed to laminar flow at high shear stress or maintained under static condition. Compared with human coronary arterial ECs exposed to laminar flow, those exposed to disturbed flow express higher levels of toll-like receptor-2 (TLR-2) (161), whose deletion in hyper-lipidemic LDLR^{-/-} mice is atheroprotective (400). The responsiveness of ECs to agonists of TLR-2 such as lipopolysaccharide and TNF- α , which are augmented in activated ECs of atherosclerotic lesions (166), is blocked by laminar flow, but not by disturbed flow, in a step-flow channel (161).

ECs exposed to low and/or reciprocating shear stress created in a parallel-plate or cone-and-plate chamber show sustained expressions or activations of NF- κ B (387, 388, 537); Sp1 (640); Egr-1 (119, 640); ICAM-1 (72, 119, 537, 640); VCAM-1 (72, 388); E-selectin (72, 119, 640); MCP-1 (257, 265); MMP-9 (200, 357); TF (368); ET-1 (474, 653); prepro-ET-1 (114); calcium/calmodulin-dependent protein kinase II (CaMKII) (64); bone morphogenic protein (BMP)-4 (536, 537) and its antagonists follistatin, noggin, and matrix Gla protein (71); gp91^{phox} [a major component of NAD(P)H oxidase complex] and its member Nox-4 (258, 266); sphingosine kinase-1 (SphK1, an enzyme that catalyzes the conversion of sphingosine to sphingosine-1 phosphate to play inflammatory and proliferative roles) (77); p21-activated kinase (PAK, a Ser/Thr kinase that modulates small GTPases Rac and Cdc42-induced cytoskeletal remodeling) (440); cathepsin K (a lysosomal cysteine ECM protease) (461); Shc (350), c-Jun-NH₂-terminal kinase (JNK) (229); PDGF-DD (a novel mediator of SMC phenotype) (566); and HuR (a stress-sensitivity regulator gene that plays inflammatory roles) (487), as well as elevations of oxidative stress or ROS levels due to continued increases of intracellular superoxide and NADH oxidase activity (138, 139, 258, 374, 526, 536). The upregulation of virtually all of these atherogenic or proinflammatory genes in ECs in response to low or reciprocating flow (with little mean flow rate) are either absent or transient in cells subjected to steady or pulsatile laminar flow with a positive mean flow rate, which may even cause opposite effects (downregulation); these upregulations are also not seen in ECs maintained under static conditions (14, 290, 321). Moreover, steady laminar flow upregulates the expression or activity of cytoprotective genes/proteins including the complement-inhibitory proteins clusterin (583) and CD59 (296), antioxidant enzymes superoxide dismutase (SOD) (570), heme oxygenase-1 (HO-1) (78, 254), NAD(P)H quinone oxidoreductase 1 (NQO1) (254), peroxiredoxins (PRX) (395), mitogen-activated protein kinase (MAPK) phosphatase (MKP-1) (642), eNOS (23, 44, 136, 258, 474, 570) and its essential cofactor tetrahydrobiopterin (615), and angiotensin-2 (Ang-2) (574), as well as induces productions of PGI₂ (184, 474), NO (56, 431, 582), and intracellular glutathione (GSH) (397), which are considered anti-atherogenic.

Laminar flow induces EC phosphorylation of GTP cyclohydrolase I (GTPCH-1), which is the rate-limiting enzyme involved in de novo biosynthesis of tetrahydrobiopterin and undergoes negative-feedback regulation by tetrahydrobiopterin via interaction with GTP cyclohydrolase feedback regulatory protein; this mechanism may be involved in the flow regulation of EC NO production (335). In contrast, reciprocating flow does not cause the inductions of CD59, HO-1, PRX, and eNOS, nor the production of NO and PGI₂ (199, 296,

527, 653), and it decreases intracellular GSH (397) and GTPCH-1 phosphorylation (335). Some of the protective effects of laminar shear stress are mediated through the inhibition of cytokine-induced signaling and gene expression in ECs (38, 89–92, 422, 445, 554, 578, 579, 633, 634) and EC apoptosis (127, 154–156, 202, 249, 459), as well as the modulation in SMC phenotype (577). The HO-1 expression in ECs induced by statin (a 3-hydroxy-3-methylglutaryl-coenzyme A reductase antagonist that exhibits lipid-independent immunomodulatory, anti-inflammatory, and cholesterol-lowering properties) is enhanced by laminar shear stress, but markedly attenuated by reciprocating flow (12). Disturbed flow increases the activation and splicing of X-box binding protein-1 (XBP-1), a key signal transducer in endoplasmic reticulum stress response; overexpression of spliced XBP-1 induces apoptosis of ECs and endothelial loss from blood vessels during ex vivo cultures because of caspase activation and downregulation of VE-cadherin resulting from transcriptional suppression and matrix metalloproteinase-mediated degradation (647). Conklin et al. (101) demonstrate that low shear stress results in significant increases in EC mRNA and protein expressions of vascular endothelial growth factor (VEGF), an endothelial-specific mitogen that increases vascular permeability and is present in human atherosclerotic lesions, compared with the physiological levels of laminar shear stress. These changes in VEGF expression may suggest a possible molecular mechanism for increased endothelial permeability in regions of disturbed flow with low shear stress in addition to the changes in junction proteins discussed above. Moreover, the increases in calcium influx and chloride current induced in EC monolayer by steady and pulsatile laminar flows are not seen with reciprocating flow (204, 244). Flow-activated ion currents exhibit different sensitivities to shear stress magnitude and oscillation frequency (341).

Studies using genomic approaches have further demonstrated that different flow patterns or shear stress distributions differentially regulate EC gene expression (50, 51, 60, 115, 201). Thus disturbed flow or shear stress applied with an athero-prone waveform replicating those in regions of disturbed flow in vivo leads to the upregulation of several pro-atherogenic genes. In contrast, exposure to laminar flow or shear stress with atheroprotective waveform increases the expression of sets of atheroprotective genes (50, 51, 60, 75, 115, 116, 201, 370, 652). Of great interest is the induction of the atheroprotective transcription factor Krüppel-like factor (KLF)-2 in ECs by atheroprotective flow waveform. KLF-2 is downstream of the MAPK ERK5 (444), which appears to play a central role in the expression of atheroprotective genes and maintenance of atheroprotective quiescent endothelial phenotype (143–145, 346, 444, 509). Young et al. (638) have demonstrated that flow-induced KLF-2 expression is mediated by the activation of AMPK. Other studies have identified nuclear factor erythroid 2-like 2 (Nrf2) as a shear-induced transcription factor responsible for antioxidant gene expression (78, 116, 254). Athero-prone and atheroprotective flow waveforms differentially regulate EC redox state and antioxidant potential through the phosphoinositol 3-kinase/Akt-dependent activation of Nrf2 and its downstream transcriptional targets (116). KLF-2 enhances the nuclear localization of Nrf2, and the combined actions of KLF-2 and Nrf2 constitute ~70% of the shear stress-induced gene expression in ECs (45, 179).

There are several epigenetic mechanisms that may modulate the gene expression and cellular function in response to various forms of shear stress. For example, recent evidence suggests that histone deacetylases (HDACs), which are a class of enzymes that regulate the structure and function of chromatin and some transcription factors and hence gene transcription, are involved in shear pattern-mediated gene expression and function in ECs. Exposure of ECs to laminar shear stress at 24 dyn/cm² induces phosphorylation-dependent nuclear export of HDAC5, which is involved in shear inductions of KLF-2 and eNOS (601). On the other hand, exposure of ECs to disturbed flow created by an orbital shaker induces serine/threonine phosphorylation of HDAC3, which serves as a pro-survival molecule with a

critical role in maintaining endothelial integrity (643). Shear stress induction of *c-fos* gene in ECs is mediated by shear-induced acetylation and phosphoacetylation of histone H3 via p38 MAPK- and protein kinase A (PKA)-dependent pathways (270). In addition, the recently discovered microRNAs, which bind to messenger RNAs to interfere with their translational process, play a significant role in the regulation of gene and protein expressions and hence a variety of biological processes (430, 551, 585). Recent studies by Qin et al. (473) and Wang et al. (599) demonstrate that application of laminar shear stress (12 dyn/cm²) to ECs regulates the EC expression of many microRNAs, including microRNA-19a and -23b, which are involved in flow regulation of EC cell cycle or growth. However, there is a lack of research on microRNAs under disturbed flow, and studies on this important topic are needed.

E. Effects of Disturbed Flow on EC-WBC Interactions

Adhesion of circulating WBCs to and their subsequent transmigration across the EC monolayers are critical events leading to vascular disorders such as inflammation and atherosclerosis (197, 491, 493). In vivo, both the local fluid dynamics of arterial flow (364) and the SMCs located in close proximity to ECs (646) exert significant influences on WBC recruitment into the vessel wall. Since the 1970s, studies by Goldsmith, Karino, and others have contributed to the understanding of fluid dynamic and rheological bases of complex blood flow in arteries resulting from the interplay of blood components and vessel geometry (85, 283, 284). Studies using the step-flow channel have shown that the adhesion of monocytes on ECs occurs preferentially in the vicinity of the reattachment point in disturbed flow (28, 88) (Fig. 16). In contrast, sustained laminar flow with a high shear stress inhibits the cytokine-induced signaling and gene expression and the adhesion of monocytes to activated ECs (88, 633, 634). The preferential distribution of monocyte-EC adhesion in regions of disturbed flow may be attributable to 1) the altered expression of adhesive proteins such as ICAM-1 and E-selectin on EC surfaces (88) and/or 2) the enhanced collisions and prolonged contacts between the circulating monocytes and ECs, as a result of a higher near-wall concentration, a longer residence time, a greater normal (perpendicular) velocity component towards the wall, and a smaller tangential velocity component along the wall in these regions (28, 88). By coculturing ECs with SMCs in collagen gels, Chen et al. (76) have demonstrated that the adhesion and transmigration of monocytes, neutrophils, and peripheral blood lymphocytes is increased by disturbed flow, particularly in the reattachment area, and that these cells exhibit different migratory behaviors beneath the EC monolayers (Fig. 17). In addition, neutrophils, peripheral blood lymphocytes, and monocytes all roll more slowly and transmigrate more rapidly in regions near the reattachment point compared with other areas. Coculture of ECs with synthetic-phenotype SMCs induces the EC expressions of various adhesion molecules and chemokines that contribute to the enhancements of WBC adhesion to and transmigration across the cocultured ECs (76). These findings demonstrate the importance of the dynamic flow environment and the underlying SMCs in modulating interactions between the circulating WBCs and the ECs, which may contribute to the regional propensity for WBC recruitment in disturbed flow areas.

F. Effects of Temporal Gradient of Shear Stress on EC Signaling and Gene Expression

In addition to the spatial gradient of shear stress at vessel bifurcations and in the step-flow channel, the temporal gradient of flow also regulates EC functions. In contrast to the inductions of ERK phosphorylation and *c-fos*, PDGF-A, and MCP-1 expressions by increases in flow as a step function (nearly instantaneous increase of shear stress from 0 to 16 dyn/cm², followed by steady shear for a sustained period) or an impulsive function (a 3-s impulse of 16 dyn/cm²) (24, 26), these inductions do not occur with a ramped increase in flow [shear stress smoothly transitioned from 0 to 16 dyn/cm² over 2 min (for ERK activity)

or 5–10 min (for gene expression), and then sustained for a desired period]. Such differential modulations of signaling and gene expression in ECs by varying the rate of rise of shear flow are accompanied by (and probably attributable to) the corresponding changes in transcription factors NF- κ B and Egr-1 mediated by NO (26). Furthermore, White et al. (614) have shown that the enhanced EC proliferation at the reattachment area in the step-flow channel seen with a step increase in flow does not occur in response to ramp-flow application. The cell proliferation induced by high temporal gradient in shear involves the activation of ERK in ECs (25). Application of laminar flow as a step function to an EC monolayer stained with 9-(dicyanovinyl)-julolidine, which is a fluorescent probe that integrates into the cell membrane and changes its quantum yield with the viscosity of the environment, results in a decrease in fluorescence intensity, indicating an increase of membrane fluidity (233), which in turn leads to the activation of heterotrimeric G proteins (227). By using a fluorescence recovery after photobleaching technique to measure lipid lateral diffusion coefficient of EC plasma membranes labeled with 1,1'-dihexadecyl-3,3',3''-tetramethylindocarbocyanine perchlorate, Butler et al. (62) demonstrate that the increase in EC membrane fluidity and the consequent ERK and JNK activation induced by the application of laminar shear as a step function do not occur with ramp flow. Thus the plasma membrane lipids are receptive to fluid shear stress (61, 62, 233). Using the isolated vessel ex vivo perfusion system, Butler et al. (63) have shown that the initial potent and transient vasodilation induced by flow is dependent on the ramping rate of the shear flow and that the second phase of modest and sustained vasodilation is dependent on the shear magnitude. All these findings indicate that temporal gradient in shear stress plays important roles in modulating signaling and gene expression in ECs, and consequently their functions.

V. IN VIVO EXPERIMENTAL MODEL STUDIES ON THE EFFECTS OF DISTURBED FLOW ON VASCULAR ENDOTHELIUM

A. In Vivo Experimental Models of High and Low Shear Stresses

In contrast to the many in vitro studies on the effects of disturbed flow on ECs, there have been limited numbers of in vivo investigations on EC responses to disturbed flow, mainly due to the lack of appropriate in vivo models that can generate well-controlled flow patterns and the difficulty of determining the molecular and cellular responses in vivo. Nevertheless, several animal models have been created to study the effects of high and low shear stresses on EC/vascular responses. The commonly used models of high shear stress in studies on rabbits and rats include 1) the creation of an arteriovenous fistula, often using carotid or femoral arteries (309, 365, 411, 516, 518, 575); 2) ligation of one of the carotid arteries to induce increases in flow and shear stress in the contralateral carotid artery (252, 300, 306, 365, 378, 624); and 3) transposition of a vein graft into the arterial circulation, thus increasing the shear stress, as well as pressure, on the ECs in the grafted segment from venous to arterial levels (271, 273, 301). Studies using these models have demonstrated that 1) the flow-induced arterial dilatation is accompanied by an adaptive remodeling of the intima and requires early expression of MMPs to degrade the ECM; 2) the NO released from ECs contributes to the increase in vessel caliber in response to a chronic increase in blood flow; 3) high shear stress causes the release of multiple vasoregulators by ECs in addition to NO, including furin, which is a yeast Kex2-family endoprotease that converts many vasoregulatory propeptides including pro-transforming growth factor (TGF)- β to their mature forms, to modulate the functions of ECs and SMCs; and 4) increases in shear stress induce the expression of heat shock protein 60 (252).

Larger animals such as baboons, sheep, and dogs have also been used for similar in vivo investigations on EC responses to alterations in shear stress (99, 302, 303, 309). The models of low shear stress include the decrease in blood flow by 1) ligation of the outflow branches

(e.g., the ipsilateral internal carotid artery) or 2) closure of an arteriovenous fistula in the rabbit or rat between the common carotid artery and the external jugular vein (29, 95, 218, 291, 300, 306, 309, 312, 378, 517, 518, 596, 624). These model studies have demonstrated that the reduction in blood flow and shear stress 1) induces EC apoptosis and death, which may affect the arterial remodeling; 2) reduces the flow-dependent enlargement of fenestrae in the internal elastic lamina of the arteries, which contributes to the internal elastic lamina remodeling; 3) leads to early neointimal hyperplasia, probably due to increased SMC migration and proliferation, monocyte adhesion, macrophage infiltration, upregulation of VCAM-1, PDGFs, and MMP-9, and ECM reorganization; and 4) induces increases in vascular oxidative stress and hence progression of experimental atheroma and angiogenesis. A recent study by Nam et al. (410) has reported that partial carotid ligation results in disturbed flow, which causes upregulation of pro-atherogenic genes, downregulation of atheroprotective genes, endothelial dysfunction, and rapid development of atherosclerosis in 2 wk and advanced lesions by 4 wk in apolipoprotein E-deficient (ApoE^{-/-}) mice.

B. Creation of Disturbed Flow In Vivo by Introducing a Stenosis in a Segment of a Large Artery

One of the most common strategies to create disturbed flow pattern and the associated low and reciprocating shear stress in vivo is to introduce a local stenosis in a segment of a large vessel, such as the carotid artery or abdominal aorta in rabbits or rats. Using this strategy to create a stenosis throat (40–60% reduction in diameter), Hutchison (264) found that ECs are aligned and elongated in the direction of flow up to five diameters upstream of the stenosis throat, where the flow is laminar with a relatively higher shear stress. Immediately downstream to the stenosis throat is a region of flow separation and low-velocity recirculation (identified by transcutaneous pulsed Doppler velocimetry), and the ECs there are maximally rounded. By five diameters downstream, the ECs gradually return to the elongated shape as observed upstream. Similar results have been reported earlier by Levesque et al. (332) and Kim et al. (293), who show that in the high-shear region immediately upstream of the stenosis throat, ECs are much more elongated, and stress fibers are markedly thicker and longer than in control aortas, and that in the region of low and reciprocating shear immediately downstream of the stenosis, ECs are polygonal in shape and show a prominent peripheral band of microfilaments. Gabriels and Paul (195) report that the expression of Cx43 is locally upregulated downstream to the stenosis, suggesting that Cx43 expression can be modulated by changes in hemodynamic pattern in vivo. Miao et al. (379) demonstrate that VE-cadherin is highly expressed at EC borders in the vessel segment at sites where the blood flow is laminar; in contrast, there is little or no VE-cadherin expression in the EC borders in the poststenotic dilatation sites, where the flow is disturbed (Fig. 18). With the same experimental model, Wang et al. (600) show that KLF-2 is highly expressed in ECs in regions of laminar flow (upstream and far downstream to the stenotic throat, as well as within the throat), but there is virtually no KLF-2 expression in the ECs at the poststenotic sites with disturbed flow (Fig. 18). McCue et al. (371) have shown that the EC polarity is age- and vessel-specific in vivo and is regulated by disturbed flow. The microtubule-organizing centers (MTOCs, from which microtubules emanate) in ECs are distributed predominantly downstream of the nuclei in abdominal aortas of immature rabbits (5-wk-old) and upstream of nuclei in mature rabbits. Following the creation of a 50% stenosis of the aorta, the flow disturbance in the recirculation eddy downstream to the stenosis eliminates these EC MTOC polarities in both immature and mature rabbits, suggesting that polarization of endothelial MTOCs in vivo is shear-dependent. These results indicate that EC structure and gene and protein expressions can be modulated by different flow patterns in vivo in a manner similar to that observed in vitro.

Cheng et al. (83) developed a perivascular shear stress modifier (a conical device with a tapered lumen, referred to as a cast) that can induce defined variations in shear stress patterns in vivo in a straight vessel segment of rabbits and transgenic mice, in which the expression and distribution of eNOS in the arterial EC can be monitored by fusion with green fluorescent protein. Placement of the cast around the carotid artery generates lowered unidirectional shear stress upstream from the cast and a downstream region of disturbed flow with reciprocating shear stress (Fig. 19A). The progressive stenosis of the vessel by the cast leads to a gradual increase in shear stress in the stenotic zone, reaching a maximum of 25 dyn/cm² at the narrowest portion (83). A strong induction of eNOS is found in the EC membrane and Golgi complex in the high-shear region around the narrowest portion of the stenotic zone, compared with the low-shear, disturbed flow regions and also the control carotid arteries that are not subjected to the treatment. The low and reciprocating shear regions show a low eNOS mRNA expression of one-third of that in the region subjected to high shear stress. En face immunostaining with antibodies against phospho-eNOS further demonstrates that the levels of eNOS phosphorylation in ECs in the high-shear region of the carotid arteries are significantly higher than those in the other regions. By using the same device, Cheng and co-workers (81, 82) have further demonstrated that, in addition to low and reciprocating shear stress in regions downstream to the device, low but unidirectional laminar flow in regions upstream to the device is also atherogenic. They found that atherosclerotic lesions develop under conditions of both unidirectional flow with low shear stress and disturbed flow with reciprocating shear stress within 6 wk of cast placement, whereas no lesions develop in the high shear stress region (Fig. 19B). The low unidirectional shear stress upstream of the cast induces the development of extensive lesions with a more inflammatory and vulnerable- plaque phenotype, whereas disturbed flow with low and reciprocating shear stress downstream of the cast induces the growth of stable lesions. Using angiotensin II administration, Cheng et al. (82) have shown that intraplaque hemorrhages occur exclusively in the atherosclerotic lesions of low unidirectional shear stress regions. However, this difference should be interpreted with caution, since one would predict that with a severe constriction, the upstream region would have higher blood pressure than the downstream region (242), and hence one cannot interpret these results solely in terms of flow patterns. In any event, reduction in flow and shear stress is associated with sustained inflammatory signaling in ECs, as well as their failure to align with flow.

In *tie1-lacZ* transgenic mice, Porat et al. (469) have shown that the expression of the *tie1* promoter in the arterial tree is enhanced in vascular bifurcations and branch points, i.e., regions of disturbed flow. Flow disturbance induced by the surgical interposition of a vein into an artery leads to an induction of *tie1* expression in the region immediately downstream of the artery-to-vein junction, where the lumen enlargement of the grafted vein due to exposure to arterial pressure provides a geometry analogous to poststenotic dilatation. This *tie1* induction by flow disturbance in vivo is confirmed by in vitro study using a step-flow channel, where the upregulation of *tie1* promoter activity is seen only in ECs residing in the region of flow separation and recirculation downstream to the step (469).

C. In Vivo Observations of EC Signaling and Gene Expression at Athero-prone Areas

Most of the in vitro studies using flow chamber on the effects of disturbed flow on EC signaling, gene expression, and functional modulation have been confirmed by in vivo observations at athero-prone regions of the arterial tree, e.g., carotid bifurcations, aortic arch, and curvatures and tortuosities of coronary arteries (Table 3). For example, ECs in athero-prone areas, i.e., regions of disturbed flow with low and reciprocating shear stress, show increases in mitotic rate (86), lipid uptake (507), and monocyte adhesion (596), which are accompanied by the changes in EC morphology and structure (57) and increases in the expression or activation of ICAM-1 (112, 168, 269, 407, 537, 552), VCAM-1 (168, 269,

407, 552, 596) (Fig. 20), E-selectin (168), PDGFs and their receptors (309, 566, 616), Cx43 (195, 314), Egr-1 (369), Ang-2 (574), cathepsin K (461), NF- κ B (48, 100, 234, 620), MMPs (29, 218), p70S6K (228), TLR-2 (399), PAK (437, 440), Shc (350), JNK (229), XBP-1 (647), HuR (487), GTPCH-1 (335), and HDAC3 (643), and decreases in AMPK (228) and Nrf2 (116), compared with the straight segments, where the blood flow is laminar and unidirectional. MCP-1 protein expression examined by immunocytochemistry has shown a preferential expression near the intercostal artery orifices compared with the straight part of the aorta (86); mapping of monocyte distribution in the aorta also shows that there is a preferential localization at branch points (362). Both BMP-2 and BMP-4, which are induced by low and reciprocating shear stress (537), are found in atherosclerotic plaques and calcified aortic valves (46, 151, 389), suggesting their importance in the development of atherosclerotic diseases. ECs overlying foam cell lesions express BMP-4, but nondiseased ECs do not (537). Interestingly, BMP-4 antagonists follistatin, noggin, and matrix Gla protein are also highly expressed in mouse aortas and human coronary arteries (71). Chronic infusion of BMP-4 causes endothelial dysfunction in a vascular NADPH oxidase-dependent manner and induces hypertension in mice (385). BMP-4 stimulates the expression and activity of NADPH oxidase through p47^{phox} and Nox-1 in an autocrine-like manner (536, 537), leading to ROS production, NF- κ B activation, ICAM-1 expression, and subsequent increases in monocyte adhesion to ECs (279, 536, 537).

ECs rely on specific ECM-integrin interactions to mediate both “inside-out” and “outside-in” signaling communications with ECM (521, 522). For example, the integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ interact with collagen, whereas $\alpha_5\beta_1$ and $\alpha_v\beta_3$ bind to fibronectin. There is little fibronectin or fibrinogen beneath the endothelium in most of the vasculature, but these proteins are found at sites of disturbed flow in vivo (439). Deletion of an alternatively spliced domain of fibronectin that reduces its assembly into matrix decreases atherosclerosis in hypercholesterolemic ApoE^{-/-} mice (562). Thus ECM remodeling with fibronectin and/or fibrinogen deposition in disturbed flow regions may induce an activated EC phenotype in these regions and promote atherosclerosis. In concert with these in vivo findings, in vitro studies have shown that the activations of NF- κ B (439), PAK (440), and JNK (229) at the onset of laminar flow and/or long-term reciprocating flow is ECM-dependent, with enhanced activity in ECs seeded on fibronectin or fibrinogen, rather than basement membrane protein laminin or collagen. Higher levels of phosphorylated NF- κ B, PAK, and JNK are present in athero-prone than in atheroprotective areas of C57BL/6J and ApoE^{-/-} mice (229, 437, 439, 440). Knockout of JNK2, but not JNK1, decreases lesion development in ApoE^{-/-} mice (488). On the other hand, injecting mice with the PAK-Nck inhibitory peptide reduces vascular permeability at athero-prone sites (440). Inhibiting PAK in ECs on fibronectin blocks the NF- κ B and JNK activations by both laminar and reciprocating flows in vitro or at sites of disturbed flow in vivo, suggesting that NF- κ B and JNK activations by flow are mediated by PAK (229, 437). In addition, these flow activations of NF- κ B and JNK in ECs on different ECM proteins are mediated by different integrins. Thus, while the flow activation of NF- κ B is prevented by the ligation of $\alpha_2\beta_1$ integrin on collagen through a p38 MAPK-dependent pathway (439), the flow activation of JNK in ECs seeded on fibronectin is inhibited by blocking $\alpha_v\beta_3$ integrin/fibronectin signaling with anti- $\alpha_v\beta_3$ monoclonal antibody LM609 (275) or anti-fibronectin antibody 16G3 (229). A mechanosensory complex consisting of the immunoglobulin family receptor platelet endothelial cell adhesion molecule (PECAM)-1, the tyrosine kinase VEGF receptor-2 (VEGFR-2), and VE-cadherin at the endothelial junctions may serve as an upstream signaling complex for ECM-integrin activation in response to flow (229). Through this complex, flow activates phosphatidylinositol (PI) 3-kinase, and the increased level of PI lipids then changes the affinity of integrins such as $\alpha_5\beta_1$ and/or $\alpha_v\beta_3$.

The signaling induced by integrin activation must be transmitted into cells through the activation of integrin-associated proteins such as Shc, an adaptor protein containing a COOH-terminal Src homology domain-2 domain, which subsequently activates several intracellular signaling cascades, including JNK and p38 MAPK (522). Shc activation occurs *in vivo* and correlates with areas of disturbed flow and atherogenesis (350). The association of Shc with integrins requires VE-cadherin, and the activation of Shc requires VEGFR-2 and Src, a tyrosine kinase that localizes to EC junctions in response to mechanical force (350, 602). Moreover, Shc regulates flow-induced ICAM-1 and VCAM-1 by activating NF- κ B-dependent signals that lead to atherogenesis (350). A recent study by Funk et al. (193) on human and bovine aortic ECs shows that basement membrane proteins enhance the flow-induced activation of PKA, which suppresses PAK. Activating PKA by injecting the prostaglandin 2 analog iloprost reduces PAK activation and ICAM-1 and VCAM-1 expressions at sites of disturbed flow *in vivo* (i.e., the outer wall of the carotid sinus in C57BL/6J mice), whereas inhibiting PKA inhibition stimulates PAK activation and inflammation *in vivo*. Thus basement membrane proteins inhibit proinflammatory signaling in ECs via PKA-dependent inhibition of PAK (193). Taken together, these results indicate that integrin-ECM is mechanosensitive and that the types of ECM in the subendothelial space would determine the responsiveness of ECs to the imposed flow patterns (521). In addition, a dynamic interaction between integrins and their cognate ECM proteins is a very early event in mechanotransduction in ECs responding to different patterns of flow and associated shear stress (521).

Endothelial glycocalyx, a carbohydrate-rich layer that is a network of membrane-bound proteoglycans and glycoproteins lining the luminal side of endothelium, may serve as a mechanosensor to transmit mechanical forces into biochemical signals in ECs (181, 470, 608, 610, 635). In healthy mice, a thinner glycocalyx is found in the internal carotid sinus region compared with the common carotid artery (73 ± 36 vs. 399 ± 174 nm) (484, 586). After 6 wk of atherogenic diet, the glycocalyx size is significantly decreased in the mouse carotid artery; at the same time, evidence of enhanced atherosclerotic plaques are observed in these mice (586). Impaired barrier properties of glycocalyx contribute to the enhanced LDL accumulation in intima at the carotid artery bifurcation in mice (587). These results suggest that flow patterns and associated shear stresses may play important roles in modulating glycocalyx dimensions and that endothelial glycocalyx may serve an atheroprotective function (428, 484). *In vitro* studies on ECs treated with heparinase III to specifically break down heparan sulfate proteoglycans in the glycocalyx (181) and *ex vivo* examinations on canine femoral arteries infused with hyaluronidase to degrade hyaluronan glycosaminoglycans in the glycocalyx (386) show significant reductions in shear-induced NO production, indicating that both heparan sulfate and hyaluronan in the endothelial glycocalyx are critical for sensing flow-induced shear forces on ECs. Interestingly, exposure of ECs to laminar shear stress (10 dyn/cm^2) increases the amount of glycocalyx hyaluronan by twofold of static controls, which may represent a positive feedback for shear stress sensing by ECs (221).

An additional mechanosensor that is regulated by different flow patterns is endothelial primary cilium, which is an integral component of apical membrane organelles on ECs. Endothelial primary cilium is present in areas of low and disturbed flows in the embryonic cardiovascular system (588) and the adult aortic arch of wildtype C57BL/6J and ApoE^{-/-} mice (589). Placement of a restrictive cast on the common carotid artery to create low and disturbed flow regions results in the presence of primary cilia only at these sites (589). In the early stage of arterial development, a shear-dependent distribution of primary cilia is present, coinciding with the expression of shear-related genes, including KLF-2, ET-1, and eNOS (225, 588). These genes respond to alterations in vascular shear stress in embryonic and adult models (80, 83, 226). In the heart, ciliated endocardial cells are only present in

areas of disturbed flow, such as the atrial and ventricular chambers, and are absent from high-shear areas such as the atrioventricular canal and outflow tracts from the ventricles (462). These results suggest that the mechanosensing proteins in the cilia may play important roles in modulating signaling and gene expression in ECs and the consequent modulation of EC/vascular functions in response to disturbed flow.

The expression of complement inhibitory protein CD59 on vascular endothelium is significantly higher in the athero-resistant regions of the murine aorta exposed to unidirectional laminar shear stress, compared with the athero-prone areas exposed to disturbed flow (296); this is significant because complement activation may predispose blood vessels to injury and atherogenesis (508). Protein kinase C (PKC)- activity has been shown to correlate with flow patterns in pig arteries, being higher in ECs exposed to disturbed flow than to unidirectional laminar flow (356). MKP-1 has been found by immunostaining to be preferentially expressed by ECs in a highshear, atheroprotected region of the mouse aorta, and to be necessary for the suppression of EC activation at this site, since its genetic deletion enhances p38 activation and VCAM-1 expression (642). Nitrotyrosine, which is considered to be an emerging inflammatory marker for atherosclerosis and a fingerprint of peroxynitrite (a product of superoxide anion reacting with NO), shows positive immunostaining in human coronary artery bifurcations or curvatures, but absence in the straight segments (258, 515). Connective tissue growth factor (CTGF), a potent angiogenic, chemotactic, and ECM-inducing growth factor, is overexpressed in atherosclerotic arteries (433) and strongly upregulated by nonuniform shear stress in ECs (637), suggesting that disturbed flow at arterial bifurcations may induce EC CTGF expression to contribute to the progress of atherosclerotic lesions. Phosphorylated nuclear activated transcription factor-2 (ATF-2), which is one of the heterodimeric components of transcription factor activator protein-1 and is crucial for cytokine-induced expression of E-selectin in ECs (90, 383, 480), is present in human ECs overlying atherosclerotic plaques at higher levels than ECs at unaffected sites; in cultured ECs, the ATF-2 activation in the EC nuclei is inhibited by laminar shear stress (180). Endothelial vWF shows substantial heterogeneity in its distribution throughout the vasculature (479), with higher levels found at sites such as branch points, bifurcations, and curvatures (19, 479, 629).

In concert with the reduced KLF-2 expression in ECs at sites of disturbed flow, as presented for in vivo and in vitro model systems (see sects. **IVD** and **VB**; Fig. 18), decreased KLF-2 expression is found at the human aortic bifurcations and the branch points of iliac and carotid arteries, coinciding with the sites of neointima formation (145). The direct involvement of shear stress in the in vivo expression of KLF-2 is demonstrated by in situ hybridization and laser microbeam microdissection/reverse transcriptase- polymerase chain reaction in a murine carotid artery collar model, in which a 4- to 30-fold induction of KLF-2 occurred at the high-shear sites (145). Lee et al. (329), using zebrafish and KLF-2-deficient mouse models, demonstrate that KLF-2 expression during development mirrors the rise of fluid shear forces and that endothelial loss of KLF-2 results in lethal embryonic heart failure. Fluid shear stress drives expression of endothelial KLF-2 that, in turn, regulates smooth muscle tone in the developing embryo (329). These results suggest that KLF-2 is an essential regulator of vascular hemodynamic forces in vivo and that hemodynamic regulation in response to fluid shear stress is required for cardiovascular development and function.

To discriminate the gene expression profiles of ECs between sites of disturbed flow and laminar flow in vivo, Passerini et al. (450) have conducted DNA microarray study on fresh ECs isolated from regions of inner aortic arch (areas exposed to disturbed flow) and descending thoracic aorta (areas exposed to laminar flow) of normal adult pigs. In the inner

aortic arch, there is an upregulation of several inflammatory cytokines and receptors, as well as elements of the NF- κ B system, which reflects a proinflammatory phenotype. This proinflammatory phenotype is probably mediated by PECAM-1, because PECAM-1-knockout mice do not exhibit activation of NF- κ B and the downstream inflammatory genes in regions of disturbed flow (239, 581). Goel et al. (219) have shown that PECAM-1 has both atherogenic and atheroprotective effects, but that the former dominates in the inner curvature of the aortic arch, whereas the latter dominates in the aortic sinus, branching arteries, and descending aorta. In this connection, the regional DNA microarray study by Passerini et al. (450) has shown that some of the atheroprotective profiles found primarily in laminar flow regions are also seen in disturbed flow regions, notably the enhanced expression of antioxidant genes. Thus the gene expression profile in disturbed flow regions may reflect a delicate balance between atherogenic and atheroprotective genes; the action of risk factors may tip the balance to initiate atherogenesis (450).

D. Experimental Model Studies on Venous Inflammation and Thrombosis Induced by Disturbed Flow With Venous Hypertension

Venous reflux through incompetent venous valves or stasis is a major cause of venous hypertension that underlies many, and possibly all, clinical manifestations of primary chronic venous diseases. There are at least three types of animal models that have been used to investigate the mechanisms by which the disturbed flow (i.e., reflux or stasis) associated with venous hypertension triggers inflammatory processes in chronic venous diseases (35). These models include 1) occlusion of a mesenteric venule in rats to allow direct observation of events occurring early after the onset of disturbed flow with increased venous pressure (35, 556–558); 2) placement of an arteriovenous fistula between the femoral artery and vein in the groin of the rat to investigate the effects of disturbed flow and venous hypertension over a longer time scale and in larger veins (35, 447, 449, 559, 560); and 3) ligation of large veins (e.g., inferior vena cava, common femoral veins, and common iliac veins) to investigate the link between disturbed flow with venous hypertension and the skin changes and thrombosis that are important manifestations of severe chronic venous diseases (35, 36, 231, 232, 320).

The venular occlusion experiments show that reduction in flow and shear stress can rapidly set in motion an inflammatory cascade, including hallmarks like WBC adhesion to the endothelium and migration into the interstitium, ROS production, and parenchymal cell death that begins immediately after occlusion and continues for at least 1 h after the release of the occlusion and reperfusion (35, 558, 619). Occlusion of a mesenteric venule also induces a significant increase of pressure upstream of the occlusion, which enhances the magnitude of the inflammatory reactions (35, 558). The formation of a femoral arteriovenous fistula induces an immediate increase in venous pressure followed by a detectable reflux, which mirrors the progressive changes in venous valve morphology and leads to, in some cases, the complete disappearance of valvular structures (35). Pressurized valves show increased numbers of granulocytes, monocytes/macrophages, and T lymphocytes and elevated levels of P-selectin and ICAM-1 in the endothelium compared with control valves. The expression of MMP-2 is increased mostly in the subintima and the media of the venous wall and in the subendothelial connective tissue of the valvular leaflets, whereas MMP-9 is mostly expressed in the intima and subintima of both the venous wall and valvular leaflets (449). Experiments using the large vein ligation model of disturbed flow with venous hypertension show that these hemodynamic alterations result in elevated levels of tissue WBCs (231, 320), which can be prevented by prior injection of an anti-ICAM-1 monoclonal antibody (232), suggesting a role of ICAM-1 in WBC adhesion and migration in skin tissues, as well as in the walls and valves of large veins.

The inferior vena cava ligation in animals (e.g., mice, rats, cats, and baboons) to produce venous stasis induces thrombus formation and deleterious changes in venous valves and vein walls (158, 401–403, 593, 594). This stasis-mediated induction of venous thrombosis requires concomitant changes in EC function and/or activation of clotting (248). Thrombus initiation is associated with a rapid inflammatory reaction in the vein wall involving early neutrophil infiltration (157) and a later wall remodeling associated with increased MMP-9 and MMP-2 expressions (141). Animal model studies indicate the roles of P-selectin, E-selectin, TF, VEGF, basic fibroblast growth factor (bFGF), and microparticles released from WBCs in modulating stasis-induced venous thrombosis (137, 401, 404, 550, 598). For example, compared with wild-type mice, mice that are null for P-selectin, E-selectin, or TF show markedly reduced thrombus formation after inferior vena cava ligation (137, 401, 404, 550). Inhibition of P-selectin using its oral small molecule inhibitor PSI-697 reduces vein wall injury (402), and administration of an anti-P-selectin antibody or a recombinant soluble form of P-selectin glycoprotein ligand-1 (PSGL-1) inhibits stasis-induced inflammation and thrombus formation (158, 169, 593). Transplantation of wild-type bone marrow into low-TF mice does not accelerate venous thrombus formation (137). Similarly, transplantation of low-TF bone marrow into wild-type mice does not suppress venous thrombus formation. These results suggest that the stasis-induced thrombus formation in the venous macrovasculature is driven primarily by TF derived from the blood vessel wall rather than circulating WBCs (137). It has been proposed that in deep venous thrombus, TF- and PSGL-1-bearing microparticles arising from cells of monocyte/macrophage lineage can bind to either P-selectin or E-selectin on the stasis-activated ECs in regions of vessel activation or inflammation to initiate clotting cascade (8, 351). The importance of microparticles, TF, and P-selectin/E-selectin in venous stasis-induced thrombosis has also been shown in the developing thrombus in mouse arterioles induced by endothelial injury model (173). In addition, it has been demonstrated that CXCR2 and CCR2, the receptors for cysteine-X-cysteine (CXC) and cysteine-cysteine (CC) chemokines that are highly expressed in vascular ECs, play important roles in modulating stasis-induced thrombus formation in the mouse inferior vena cava ligation model (246, 247).

The results drawn from the experimental animal model studies on the role of disturbed flow in the pathogenesis of chronic venous diseases are comparable with the examinations of affected veins from patients with chronic venous diseases, which show 1) increased monocyte/macrophage infiltration of the valvular leaflets and venous wall (436); 2) elevated ICAM-1 expression in the vein wall and valves (555); 3) increased levels of ICAM-1, VCAM-1, and E-selectin in plasma, suggesting endothelial activation (274, 497); 4) greater levels of activation of circulating WBCs and elevated ROS production in plasma (561); 5) increased numbers of macrophages, lymphocytes, and mast cells in skin biopsies (443, 617); 6) elevated levels of mast cell infiltration, suggesting that inflammation may be a cause rather than a consequence of venous disease (280, 630); 7) increased ratios of TIMP-1/MMP-2 and TIMP-2/MMP-2 (3.6 and 2.1 times, respectively) and elevated levels of cytokines TGF- β 1 and bFGF in the walls of varicose veins compared with control subjects, which could favor the accumulation of ECM in varicose veins (18); 8) higher plasma levels of VEGF and PDGF compared with control subjects (380, 519); and 9) higher plasma levels of MMP-9 in patients with varicose veins in response to postural blood stasis (274). Most of these events occurring in patients with chronic venous diseases are also present in the formation and progression of atherosclerotic lesions, indicating a common role played by disturbed flow in the pathogenesis of arterial and venous diseases.

VI. ENDOTHELIAL HETEROGENEITY AND ITS PATHOPHYSIOLOGICAL BASIS FOR DISTURBED FLOW-RELATED VASCULAR PATHOLOGIES

ECs from different anatomical locations demonstrate a remarkable heterogeneity in both structure and function under normal and pathological conditions (5, 6). The notion of phenotypic diversity of ECs arises from both *in vivo* (510, 538) and cell culture studies (60, 84, 146, 567), which indicate that there are substantial differences in gene expression between ECs from different vascular sites. These differences in gene expression and phenotypes in ECs from different vascular beds may account for specific susceptibility of vascular beds to pathological conditions, including disturbed flow with altered shear stress (7). For example, while vWF is expressed at the significantly higher levels in venous ECs than arterial ECs (631), eNOS is mainly expressed in the arterial side of the circulation (15, 468). TNF- α is able to activate NF- κ B and activated protein-1 in both human umbilical artery and vein ECs, but can cause inductions of VCAM-1 and E-selectin only in human umbilical vein ECs (347). ICAM-1 is induced in both cell types, but at a higher level in umbilical venous than arterial ECs (347). Freshly isolated umbilical arterial ECs express higher levels of KLF-2 than umbilical venous ECs, and the fall in KLF-2 expression when umbilical arterial ECs are placed in cell culture correlates with a gain in TNF-responsiveness (347). *In vivo* studies on adhesion molecule expression and WBC adhesion in the mouse aorta and inferior vena cava using intravital microscopy and immunostaining have demonstrated that venous ECs have a greater capacity to mount an inflammatory response than arterial ECs (8, 171). It is possible that the proinflammatory properties of venous ECs influence the development of venous thrombosis and vein graft atherosclerosis in which inflammatory responses play major roles (130, 171, 593). By using DNA microarray technique to analyze 52 purified EC samples from 14 distinct locations, Chi et al. (84) have demonstrated that significant transcriptional differences exist between cultured arterial and venous ECs, e.g., arterial ECs have higher levels of CD44 and endothelial lipase, a member of the triglyceride lipase gene family, than venous ECs. This difference is in accordance with the finding that arterial and venous ECs are derived from separate sets of embryonic precursor cells with distinct gene expression profiles (327). The notion that the higher levels of CD44 and endothelial lipase in arterial ECs may underlie the preferential arterial involvement of atherosclerosis (84) is supported by the findings that disruption of CD44 in mice results in a dramatic decrease in atherosclerosis (110) and that an elevated level of endothelial lipase is associated with reduced high-density lipoprotein and increased atherosclerosis (96, 272). By exposing human saphenous vein and coronary artery ECs to venous-like (2.2 dyn/cm², steady flow) and coronary artery-like (17 \pm 1.4 dyn/cm², 1 Hz) flow patterns in a custom Silastic tube perfusion system (22), Methe et al. (377) have demonstrated that arterial and venous ECs respond differentially to different flow patterns and shear stresses. While both flow patterns have no effect on VCAM-1 and E-selectin expressions in these two types of ECs, ICAM-1 regulation by flow is more complex. Coronary artery-like flow induces a higher level of ICAM-1 expression in human coronary artery ECs than that induced by venous-like flow. In contrast, the expression of ICAM-1 in human saphenous vein ECs exposed to coronary artery-like flow is lower than that exposed to venous-like flow. However, both flow patterns induce higher levels of ICAM-1 expression in human saphenous vein ECs than in human coronary artery ECs. These results indicate that flow-dependent regulation of ICAM-1 expression differs markedly between arterial and venous ECs (377).

Endothelial heterogeneity between straight and branched parts of the arterial system or among different arteries may influence the athero-susceptibility of different arteries. For example, TF is virtually undetectable in normal endothelium in the arterial system, but in a baboon sepsis model in which lethal doses of *Escherichia coli* are intravenously injected, TF

is expressed at higher levels in the arterial branches than in the straight segments of aorta, suggesting that sitedependent endothelial heterogeneity contributes to focal pro-coagulant responses to septic stimuli under disturbed flow (8, 353). A human biopsy study by Dalager et al. (117) suggests that the initiation, development, and composition of atherosclerotic plaques differ among arteries in different regions. The coronary arteries have a higher prevalence of atherosclerotic plaques compared with the carotid and femoral arteries. ECs derived from coronary arteries of male New Zealand rabbits have significantly lower eNOS mRNA levels and a stronger pro-atherogenic gene expression pattern compared with aortic ECs (118). Zhang et al. (650) demonstrate the EC heterogeneity between typical athero-prone and athero-resistant arteries in vivo by comparing gene expression profiles of ECs isolated from freshly harvested porcine coronary versus iliac arteries with the use of DNA microarrays. The in vivo transcriptional difference between these two types of ECs has provided insights into the factors responsible for coronary artery athero-susceptibility. Systematic identification of molecular biomarkers for ECs from specific vessels or vascular beds will not only enhance our understanding of the mechanisms underlying endothelial heterogeneity of different vascular beds and its pathophysiological basis for vascular disorders, but may also provide the potential for site-specific delivery of therapeutic agents.

VII. FLOW-RELATED SIDENESS OF VALVULAR ENDOTHELIAL PHENOTYPE AND ITS PATHOPHYSIOLOGICAL SUSCEPTIBILITY

There is increasing evidence that endothelium plays a critical role in the pathogenesis of valvular heart disease and that aortic valve stenosis is associated with systemic endothelial dysfunction, with the characteristics of the atherosclerotic process (328, 463). Aortic valve diseases preferentially occur on the aortic side of the valvular leaflets where they are exposed to complex flow patterns (432, 441). However, the contributions of valvular endothelial phenotypes to this sidespecific response potentially associated with the local flow environment have not been fully clarified. The exposure of valvular ECs to unidirectional laminar shear stress results in the alignment of these ECs perpendicularly to the direction of flow, in contrast to the parallel alignment of vascular ECs to flow (58, 59). Comparison of transcriptional profiles of valvular versus vascular ECs under static and shear stress conditions shows that up to 10% of the genes considered in that study are significantly different (60), suggesting clear phenotypic differences between these two cell types in response to shear stress. Despite these differences, the pathological inflammatory responses of the two cell types involve similar mediators such as ICAM-1, VCAM-1, and E-selectin (398). In the context of the valvular response, these mediators are expressed preferentially on the aortic side of the leaflets. Using RNA amplification and DNA microarrays, Simmons et al. (529) identified 584 genes that are differentially expressed in situ by the endothelium on the aortic side versus ventricular side of normal adult pig aortic valves. These differential transcriptional profiles, representative of the steady state in vivo, identify globally distinct endothelial phenotypes on opposite sides of the aortic valve. Several inhibitors of cardiovascular calcification are less expressed by endothelium on the disease-prone aortic side of the valve, suggesting side-specific permissiveness to calcification. Sucofsky et al. (548) demonstrate the involvement of BMP- and TGF- β 1-dependent signaling events are involved in aortic valve inflammation, which occurs preferentially on the aortic side of the valvular leaflets. These ex vivo findings implicate that complex flow conditions in the aortic side of the valvular leaflets may regulate valvular endothelial signaling, gene expression, and phenotype to contribute to the preferential susceptibility to lesion development in this region.

Venous valves are frequent sites of venous thrombosis initiation. However, the possible contribution of the valvular sinus endothelium to the initiation of venous thrombosis has received little attention. Using immunofluorescence staining to examine great saphenous

veins harvested at cardiac bypass surgery, Brooks et al. (52) have demonstrated an upregulation of anticoagulant and downregulation of procoagulant activities in the endothelium of venous valve sinus (i.e., the distal side of valvular leaflets) compared with that of vein lumen (i.e., the proximal side of valvular leaflets). The expressions of endothelial protein C receptor and thrombomodulin, both of which facilitate the thrombin-induced activation of protein C in the anticoagulant and anti-inflammatory pathways, are higher in the endothelium of valvular sinus than those in the endothelium of vein lumen. In contrast, the expression of vWF is lower in the sinus endothelium than the vein luminal endothelium. These results suggest that valvular sinus endothelium may maintain a thromboresistant phenotype to play homeostatic roles in protecting against venous thrombogenesis (8, 52).

VIII. CLINICAL APPLICATIONS AND PERSPECTIVES

We have discussed in this review the physiological and pathophysiological relevance of the effects of disturbed flow and its associated shear stress pattern on vascular ECs. The results of studies on this subject have significant applications and implications in several fields of clinical medicine and surgery related to vascular diseases. The clinical applications based on these concepts include the following: 1) maintenance and enhancement of shear stress to improve endothelial/vascular functions in patients undergoing vascular surgery; 2) minimization of regions of disturbed flow created by surgical interventions and host-prosthesis mismatch; 3) augmentation of blood vessel formation by modulation of shear stress; 4) development of gene therapy strategies and antiatherogenic drugs that take into account the shear stress-responsive targets; and 5) prevention of reflux and venous hypertension to alleviate symptoms of chronic venous diseases

A. Maintenance and Enhancement of Shear Stress to Improve Endothelial/Vascular Functions

Neointimal hyperplasia is a critical process leading to atherosclerosis and restenosis, as well as their complications. Since high shear stresses with a significant net direction can result in a reduction in neointimal hyperplasia, several strategies have been designed to modify shear stress to increase vascular patency following surgical interventions such as bypass graft and stenting. Thus a distal arteriovenous fistula has been used to increase blood flow and shear stress above a critical threshold to prevent thrombosis and neointimal hyperplasia of the graft, with the goal of improving the patency of bypass graft that had poor outflow (120, 121, 442, 472). Seeding of prosthetic bypass grafts with an EC monolayer reduces thrombosis and improve patency; application of a physiological level of laminar shear stress to such grafts increases the adhesion of seeded ECs and enhances their retention after implantation in vivo, thereby improving graft patency (418, 429). Carlier et al. (67) have shown that the insertion of a flow divider (Anti-Restenotic Diffuser, Endoart) in a stent placed in the external iliac arteries of rabbits on a hypercholesteremic diet increases the shear stress to result in a local reduction in neointimal hyperplasia and the consequent prevention of in-stent restenosis. In addition to these applications of shear stress modifications to improve vascular patency following surgical interventions, there is evidence that exercise training can improve endothelial function and is beneficial to the treatment and prevention of cardiovascular diseases (98, 160, 426, 427). Exercise-induced increases in flow and shear stress also enhance the expression of eNOS and production of NO by ECs (238, 582) to increase myocardial perfusion, with the consequent retardation of progression, or even facilitation of regression, of coronary artery disease (505). In addition, the increase in shear stress and flow magnitude may move the stagnant zone and reattachment point downstream so that the athero-prone region will not be constantly exposed to the disturbed flow. This is probably why exercise needs to achieve a certain degree of intensity, maintain a certain duration of time, and sustain with a certain regime of

regularity. All of these will help to move the stagnation, reattachment points downstream away from the disease-prone areas which would have stayed at the same place in a sedentary situation.

B. Minimization of Disturbed Flow Regions Created by Surgical Interventions and Host-Prosthesis Mismatch

In vascular surgery, it is crucial to avoid mechanical and geometrical changes that may result in large flow disturbances and the consequent thrombosis and restenosis. Studies on bypass surgery have demonstrated that the graft angle at the distal anastomosis is one of the determinants of shear stress and flow pattern (113, 340, 343, 513, 539). A sharp degree of angulation contributes to the formation of complex flow patterns and areas of low shear stress within the anastomosis (113, 539), leading to thrombosis and restenosis, and hence graft failure. Several types of anastomotic cuffs or patches have been developed to reduce the compliance mismatch between the artery and the graft, with the aim of minimizing areas of disturbed flow and low shear stress, thus improving bypass graft patency (113, 178, 297, 310, 340, 381, 419, 546). When a saphenous vein graft is used, valvulotomy of venous valves before coronary artery bypass allows a more even flow and thus avoids a potential cause for thrombus formation and graft failure (382). It is beneficial to reinforce the saphenous vein prior to grafting to prevent the occurrence of mechanical mismatch between the vein graft and the host artery (86, 348). Clinical evidence has suggested that the patency rate of small grafts is improved by matching the elastic properties of the graft to that of the host artery (340). Thus it is important to consider the mechanical and geometrical matching between the graft and the host vessel to prevent the creation of complex flow patterns due to host-prosthesis mismatch. It has been repeatedly shown that there is a link among stent design (165, 167, 288), implantation strategy (10, 167, 287), and clinical outcome, and also between wall shear stress and neointimal formation, thrombosis, reendothelialization, and restenosis (298, 543, 544). The flow separation zones induced by stent struts can be reduced in size or eliminated by the incorporation of fluid dynamic theory into stent strut design, suggesting that strut streamlining will prevent complex flow conditions that would lead to local inflammation and coagulation associated with stents (277). In addition to stent deployment, cardiac surgeons and cardiologists also need to address the optimization of hemodynamic performance in aortic valve replacement surgeries.

C. Augmentation of Blood Vessel Formation by Modulation of Shear Stress

Patients undergoing bypass surgery and angioplasty tend to suffer from risks such as restenosis and neurological complications (420). To counter this problem, a new approach for the treatment of arterial occlusive disease has been proposed to restore blood flow of affected area by stimulating the growth of collateral vessels, viz. "arteriogenesis," to bypass the sites of arterial lesions (241). Shear stress plays a significant role in arteriogenesis. Under physiological conditions, almost no net blood flow is present in preexisting collateral anastomoses. Stenosis or occlusion of a major artery can induce dramatic changes in the local circulation and the local blood pressure, which tend to increase the shear stress within the collateral network as a compensatory mechanism to activate the collateral endothelium. This would lead to the recruitment of circulating blood monocytes and bone marrow-derived cells and the proliferation of vascular cells, thus facilitating arteriogenesis. In general, collateral arteries cannot completely restore the physiological level of arterial blood flow, and the growing process would stop at a stage of partial adaptation (241). Several approaches, including gene and cell therapies, have been tested with the aim of augmenting arteriogenesis (486). Novel knowledge gained from studies on EC responses to changes in shear stress may be applied to designing new biochemical and mechanical therapeutic strategies to enhance arteriogenesis (486).

D. Development of Gene Therapy Strategies and Anti-atherogenic Drugs That Take Into Account Shear Stress-Responsive Targets

Increases in our understanding of how ECs respond to disturbed flow and changes in shear stress can lead to breakthroughs in the design of novel therapeutic strategies for gene therapy and anti-atherogenic drugs. For example, Bryant et al. (54) have shown that the delivery of Egr-1, which is highly responsive to shear stress, promotes angiogenesis and tissue repair in vivo. Jin et al. (278) and Wu et al. (627) have tested the possibility of using the negative mutant of Ras (an upstream signaling molecule of MAPK), i.e., RasN17, to prevent the vascular stenosis induced by balloon injury in animal experiments. With the delivery of adenovirus carrying RasN17, the wall thickening following balloon injury is markedly attenuated, with sufficient widening of the lumen to allow an essentially normal flow (Fig. 21). Houston et al. (255) have developed hybrid vectors that contain the luciferase reporter gene with the shear stress-responsive element (with the nucleotide sequence of "GAGACC") in the promoter region. Instillation of these vectors in vivo into the left carotid artery of rabbit and subsequent generation of a stenosis using a mechanical wire occluder results in a 10-fold upregulation of luciferase reporter gene expression at the sites of vessel occlusion, compared with the unstenosed sites. These vectors show promise for use as a carrier of therapeutic genes for gene therapy of vascular occlusive disease. Laminar shear stress induces the EC expression of eNOS and productions of NO and superoxide dismutase (which reduces oxidative degradation of NO); such shear-induced effects would be beneficial since antioxidants or the NO precursor L-arginine can improve endothelial function, enhance coronary blood flow, reduce angina, and improve exercise tolerance in patients with coronary artery disease (331). These examples illustrate how basic research on EC responses to shear stress and disturbed flow can generate new approaches for the development of strategies for gene therapy and anti-atherogenic drugs for clinical management of vascular disease.

E. Prevention of Venous Stasis and Reflux to Alleviate Symptoms of Chronic Venous Diseases

Clinical treatments for chronic venous diseases generally aim to prevent venous stasis, reflux, and venous hypertension, as well as their induced inflammation, which can alleviate symptoms of chronic venous diseases and reduce the risk of ulcers, thereby improving the quality of life. Standard therapies for varicose veins are exercise, weight loss, blood pressure control, and compression stockings. Exercise with foot movements may increase the velocity of main stream of venous flow, thus reducing the pressure on the luminal side of the valvular leaflets to cause closure of the valve; this would minimize the reflux and prevent the exposure of endothelial surfaces to reverse blood flow (36). Compression stockings are clinically effective to improve venous hemodynamics and reduce edema in patients with chronic venous diseases, but they may not be usable for a wide variety of reasons, including application difficulty (because of frailty or arthritis), physical constraints (e.g., limb obesity, contact dermatitis, or tender, fragile, or weepy skin), and coexisting arterial insufficiency (268, 478). Recent advances in surgical research suggest that surgical procedures such as saphenous ablation or stent replacement to correct venous reflux or obstruction can aid rapid healing and prevent the recurrence of ulcers, with the reduction in or discontinuation of the use of compression stockings afterwards (413, 414, 478). In addition, treatment with agents that reduce oxidative stress by scavenging ROS or inhibit the inflammatory cascade may prevent the progressive deterioration of the valves and wall of the affected veins and hence reduce the reflux (35). For example, animal experiments show the occurrence of significant attenuation in the distortion of valves in affected veins and reduction in the rate of reflux in response to the administration of micronized purified fraction of flavonoid in a dose-dependent manner (35); flavonoid is a naturally occurring antioxidant substance that can scavenge superoxide anions and lipid peroxy radicals (3, 490) and reduce WBC adhesion

and migration after arterial ischemia/reperfusion (47, 191). Discovery of drugs to inhibit various elements of the proinflammatory cascade induced by disturbed flow, particularly the WBC-EC interactions that are important in many aspects of the disease, may offer the greatest opportunity to prevent chronic venous disease-related complications (163). Improved understanding of the molecular and cellular mechanisms involved in disturbed flow-mediated pathogenesis of chronic venous diseases can lead to the identification of new molecular targets for pharmacologic intervention.

IX. SUMMARY AND CONCLUSIONS

The EC monolayer, located at the interface between the flowing blood and the vessel wall, is directly exposed to blood flow and associated shear stress, and its dysfunction in response to chemical and mechanical stimuli can lead to vascular disease. Disturbed blood flow patterns (e.g., flow separation, recirculation, reattachment, stasis, and reflux) occur naturally at certain vascular regions (such as arterial branch points and curvatures) and the aortic and venous valve sinuses, as well as in pathophysiological conditions (such as atherosclerotic plaques, incompetence of venous valves and outflow obstruction, etc.) and following interventional procedures (such as end-to-side anastomosis in bypass graft surgery and stent deployment in balloon angioplasty). The flow disturbances that occurred naturally or associated with vascular diseases and interventions can lead to neointimal hyperplasia or thrombosis, which result in undesirable vascular consequences and clinical conditions, including atherosclerosis, restenosis following angioplasty, in-stent restenosis, bypass graft occlusion, transplant vasculopathy, and aortic valve calcification, as well as various chronic venous diseases. In this review, we have summarized the current state of *in vitro* and *in vivo* research on the effects of disturbed flow and the associated alterations in shear stress on ECs, in terms of signal transduction, gene expression, and cellular structure and functions (for summary, see Figs. 22 and 23). In the relatively straight portion of an artery, which is exposed to laminar flow with a physiological level of shear stress (10–70 dyn/cm²), ECs are aligned and elongated in the direction of flow, with the formation of very long, well-organized, parallel actin stress fibers in the central region and the continuous distributions of intercellular junctional proteins (e.g., Cx43 and VE-cadherin) in the periphery. In contrast, in areas of disturbed flow, i.e., the arterial branch points and curvatures, the ECs are more polygonal in appearance without a clear orientation; their actin filaments are short, randomly oriented, and localized mainly at the cell periphery, and their intercellular junctional proteins have discontinuous distributions. The accelerated cell turnover rate at regions of disturbed flow probably plays a significant role in the disruption of junctional proteins and the consequent increase in permeability to macromolecules such as lipoproteins. In these regions, there is also a sustained activation of SREBP, which acts in concert with the increase in permeability to contribute to the increase in lipid accumulation in regions of disturbed flow.

Sustained laminar shear stress in a physiological range with a definitive net direction activates signaling pathways that induce the expression of several atheroprotective and antithrombogenic genes encoding products that serve antioxidant, anti-inflammatory, anticoagulant, and antiapoptotic functions. These protective effects of laminar shear stress are mediated via a number of mechanisms, including the inhibition of cytokine-induced EC signaling, suppression of EC proliferation, and modulation of SMC phenotype toward contractile. In contrast, disturbed flow with a low and reciprocating shear stress and a high shear stress gradient induces the expression of a number of atherogenic and thrombogenic genes (pro-oxidant, pro-inflammatory, pro-coagulant, and pro-apoptotic), enhancement of EC proliferation, and modulation of SMC phenotype toward synthetic. These findings indicate that laminar shear stress in a physiological range maintains vascular homeostasis and plays protective roles against atherosclerosis and thrombosis, whereas EC dysfunction

induced by disturbed flow with low and reciprocating shear stress would predispose these vascular regions to atherogenesis and thrombosis.

Disturbed flow may also occur in the aortic and venous valve sinuses, which may lead to the sidedness of valvular endothelial phenotypes and responses that contribute to the side-specific susceptibility to calcification or thrombosis. The response of valvular ECs to shear stress may be different from that of vascular ECs, in terms of gene expression and structure. In contrast to parallel alignment of vascular ECs exposed to unidirectional laminar flow, the aortic valvular ECs tend to be perpendicularly oriented to the direction of flow. The transcriptional profile of the endothelium on the aortic side of aortic valvular leaflets is different from that on the ventricular side, with higher expression of proinflammatory factors and lower expression of calcification inhibitors, suggesting the side-specific permissiveness to atherosclerosis and calcification. The sidedness of venous valvular endothelium is evidenced by the finding that venous valvular sinus endothelium maintains a thromboresistant phenotype, with higher expression of anticoagulant and anti-inflammatory factors compared with the endothelium on the opposite side of valvular leaflets, which may play homeostatic roles in protecting against venous thrombogenesis.

The responses of venous ECs to disturbed flow resulting from reflux through incompetent valves and outflow obstruction or stasis in patients with chronic venous diseases resemble the responses of arterial ECs in atherogenesis, in terms of increased adhesion and transmigration of WBCs and elevated expressions of adhesion molecules ICAM-1, VCAM-1, and E-selectin, proteases MMP-2 and MMP-9, and mitogens VEGF and PDGF, and production of ROS at the vein wall or release into the plasma. These responses of venous ECs to disturbed flow render the veins more susceptible to inflammation, leading to the development and progression of various chronic venous diseases, including varicose veins, deep venous thrombosis, and chronic venous insufficiency. There are differences for ECs from veins and arteries. For example, venous ECs may have greater capacity to mount an inflammatory response compared with arterial ECs, and this may influence the development of venous thrombosis and vein graft atherosclerosis in which the inflammatory responses play major roles. Identification of specific biomarkers and mechanisms accounting for the endothelial heterogeneity between arteries and veins and among different vascular beds, as well as the role of this heterogeneity in the pathophysiology of vascular diseases, may provide novel approaches for site-specific therapeutic intervention for cardiovascular diseases.

Vascular biology has emerged as an important discipline in basic biomedical sciences and clinical medicine, and there is increasing recognition of the necessity of considering both mechanical and chemical factors in vascular biology in health and disease. Studies on the effects of disturbed flow on ECs have produced important information on the interplays between blood flow and vessel geometry in the development of intimal hyperplasia, atherosclerosis, thrombosis, chronic venous diseases, and their clinical complications. The application of such information to clinical medicine and surgery has emerged only recently. One example of the application is the design and improvement of reconstructive procedures that take into account the flow mechanics to optimize their success in vascular surgery. For example, the mechanical compliance and geometry of graft-to-artery distal anastomoses in bypass vascular surgery play significant roles in determining early and late graft successes/failures due to thrombosis, neointimal hyperplasia, and atherosclerosis; optimization of the factors considered in this review can significantly improve the outcome. Furthermore, investigations on EC responses to disturbed flow will provide important information concerning alterations in vascular signaling, gene expression, and function in the disease-prone regions. These results can help to develop new methods to distinguish the dysfunctional or activated ECs from healthy or quiescent ECs. Elucidation of the dynamic

interactions between disturbed flow-induced EC dysfunction and systemic risk factors, such as smoking, hyperlipidemia, hyperglycemia, hypertension, obesity, and diabetes, will enhance our understanding of the pathophysiological mechanisms of vascular diseases, thus improving the success of therapeutic modalities used to counter disease progression and improve clinical outcomes. A major challenge in this field is to conduct innovative, interdisciplinary research that combines bioengineering, physiology, other biomedical sciences, and clinical medicine to elucidate the role of disturbed flow in the pathophysiology of cardiovascular diseases, with the ultimate goal of integrating and translating the large body of information for the development of novel therapeutic strategies for these diseases.

Acknowledgments

We express our sincere thanks to Dr. Shunichi Usami for initiating the investigations on disturbed flow in our laboratories, for his valuable advice and innovative design of the step-flow channel, as well as for his precious teaching (to J.-J. Chiu) and wonderful collaboration for half a century (with S. Chien). We acknowledge the valuable help from Drs. Jing Zhou and Shun-Fu Chang and Ms. Mei-Chun Wang during the preparation of the manuscript.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-106579 and HL-104402 (to S. Chien), National Science Council (Taiwan) Grant to UST-UCSD International Center of Excellence for Bioengineering (to S. Chien), National Health Research Institutes (Taiwan) Grant ME-098-PP-06 (to J.-J. Chiu), and National Science Council (Taiwan) Grants 99-3112-B-400-008 and 99-2321-B-400-002 (to J.-J. Chiu).

GLOSSARY

Complex flow patterns	The patterns of flow that are difficult to characterize; they can be made of many different types of flow such as disturbed flow, reciprocating flow, recirculation eddy, flow separation and reattachment, etc.
Cyclic stretch	Periodic lengthening of elements in the vessel wall in the circumferential direction produced by periodic increases in transmural pressure difference, or periodic lengthening of the cultured endothelial cells in one direction (uniaxial cyclic stretch) or all directions (biaxial cyclic stretch).
Disturbed flow	The pattern of flow that is nonuniform and irregular, including recirculation eddies and changes in direction with time (reciprocating flow) and space (flow separation and reattachment).
Flow reattachment	After flow separation, the part of the flow that forms the boundary between the recirculating flow and the forward flow through the central region of the tube (vessel) is called the dividing streamline. The point at which the dividing streamline attaches to the wall again is called the flow reattachment point, where the velocity gradient and shear stress vanish to become zero.
Flow separation	The boundary layers of flow that separate from the surface of the vessel wall to form a recirculation eddy behind the separation point, where the velocity gradient and shear stress vanish.
Fluid shear stress	The tangential component of frictional forces generated at a surface (e.g., the vessel wall) by the flow of a viscous fluid (e.g., blood). The unit for shear stress is Pascal (Pa) in the SI system (the International System of Units). In the cardiovascular system, dyn/cm ² is usually

used: $1 \text{ Pa} = 10 \text{ dyn/cm}^2$. The “shear stress” used in this article denotes wall shear stress unless otherwise stated.

Hydrostatic pressure	The pressure exerted by a fluid at equilibrium due to the force of gravity.
Laminar flow	A well-ordered pattern of streamlined flow that occurs when a fluid flows in parallel layers, with friction between the successive layers.
Pulsatile flow	Periodic flow with a positive mean flow rate; the velocity of the fluid oscillates in time at the frequency of the periodicity with a significant net direction.
Reciprocating flow	Periodic flow with little mean flow rate; the velocity of the fluid oscillates in time mainly back and forth at the frequency of the periodicity.
Recirculation eddy	Swirling of a fluid with reverse streamlines created when the fluid flows past an obstacle, a curvature, or a region with diameter change.
Reflux	The reflux in the venous system is defined as a pathophysiological retrograde (distally directed) flow in an incompetent vein that occurs between the veins in the thigh and the lower leg of an individual in an erect position and under the influence of gravitation.
Retrograde flow	The flow of fluid in a direction other than the physiological direction, as in reflux.
Step flow	The flow over a backward-facing step, which is achieved in the cone-and-plate viscometer by imposing a rectangular obstacle or in the parallel-plate flow chamber by using two silicone gaskets with the top one having a longer longitudinal cutout than the one below (Fig. 8). This flow is comprised of a well-defined recirculation eddy immediately downstream of the step, followed by a region of flow reattachment, and finally with a unidirectional laminar flow that is reestablished further downstream (Fig. 9).
Turbulent flow	Flow in which the velocity at any given point varies continuously over time, even though the overall flow may be steady. In turbulent flow, the inertial forces are more significant than viscous forces; it begins to be significant when the Reynolds number (flow velocity \times fluid density \times vessel diameter/fluid viscosity) exceeds a critical level; this critical Reynolds number becomes lower with an increase in complexity of vascular geometry. Turbulent blood flow is uncommon in normal circulation, but it occurs in human aorta at peak systole (especially during heavy exercise), in arteries distal to severe stenoses, and in aneurysms.

REFERENCES

1. Abeles D, Kwei S, Stavrakis G, Zhang Y, Wang ET, García-Cardena G. Gene expression changes evoked in a venous segment exposed to arterial flow. *J Vasc Surg.* 2006; 44:863–870. [PubMed: 17012009]
2. Aboyans V, Criqui MH, Denenberg JO, Knoke JD, Ridker PM, Fronek A. Risk factors for progression of peripheral arterial disease in large and small vessels. *Circulation.* 2006; 113:2623–2629. [PubMed: 16735675]

3. Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol.* 1989; 38:1763–1769. [PubMed: 2735934]
4. Aird WC. Endothelium and allotransplantation. *Transplantation.* 2006; 82:S6–S8. [PubMed: 16829797]
5. Aird WC. Phenotypic heterogeneity of the endothelium. I. Structure, function, and mechanisms. *Circ Res.* 2007; 100:158–173. [PubMed: 17272818]
6. Aird WC. Phenotypic heterogeneity of the endothelium. II. Representative vascular beds. *Circ Res.* 2007; 100:174–190. [PubMed: 17272819]
7. Aird WC. Spatial and temporal dynamics of the endothelium. *J Thromb Haemost.* 2005; 3:1392–1406. [PubMed: 15892866]
8. Aird WC. Vascular bed-specific thrombosis. *J Thromb Haemost 5 Suppl.* 2007; 1:283–291.
9. Akimoto S, Mitsumata M, Sasaguri T, Yoshida Y. Lamina shear stress inhibits vascular endothelial cell proliferation by inducing cyclin-dependent kinase inhibitor p21 Sdi1/Cip1/Waf1. *Circ Res.* 2000; 86:185–190. [PubMed: 10666414]
10. Al Suwaidi J, Berger PB, Rihal CS, Garratt KN, Bell MR, Ting HH, Bresnahan JF, Grill DE, Holmes DR. Immediate and long-term outcome of intracoronary stent implantation for true bifurcation lesions. *J Am Coll Cardiol.* 2000; 35:929–936. [PubMed: 10732890]
11. Albuquerque ML, Waters CM, Savla U, Schnaper HW, Flozak AS. Shear stress enhances human endothelial cell wound closure in vitro. *Am J Physiol Heart Circ Physiol.* 2000; 279:H293–H302. [PubMed: 10899069]
12. Ali F, Zakkar M, Karu K, Lidington EA, Hamdulay SS, Boyle JJ, Zloh M, Bauer A, Haskard DO, Evans PC, Mason JC. Induction of the cytoprotective enzyme heme oxygenase-1 by statins is enhanced in vascular endothelium exposed to lamina shear stress and impaired by disturbed flow. *J Biol Chem.* 2009; 284:18882–18892. [PubMed: 19457866]
13. Ando J, Nomura H, Kamiya A. The effect of fluid shear stress on the migration and proliferation of cultured endothelial cells. *Microvasc Res.* 1987; 44:62–70. [PubMed: 3561268]
14. Ando J, Tsuboi H, Korenaga R, Takada Y, Toyama-Sorimach N, Miyasaka M, Kamiya A. Down-regulation of vascular adhesion molecule-1 by fluid shear stress in cultured mouse endothelial cells. *Ann NY Acad Sci.* 1996; 748:148–157. [PubMed: 7535026]
15. Andries LJ, Brutsaert DL, Sys SU. Nonuniformity of endothelial constitutive nitric oxide synthase distribution in cardiac endothelium. *Circ Res.* 1998; 82:195–203. [PubMed: 9468190]
16. Asakura T, Karino T. Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circ Res.* 1990; 66:1045–1066. [PubMed: 2317887]
17. Azumi H, Inoue N, Takeshita S, Rikitake Y, Kawashima S, Hayashi Y, Itoh H, Yokoyama M. Expression of NADH/NADPH oxidase p22^{phox} in human coronary arteries. *Circulation.* 1999; 100:1494–1498. [PubMed: 10510050]
18. Badier-Commander C, Verbeuren T, Lebard C, Michel JB, Jacob MP. Increased TIMP/MMP ratio in varicose veins: a possible explanation for extracellular matrix accumulation. *J Pathol.* 2000; 192:105–112. [PubMed: 10951407]
19. Badimon L, Badimon JJ, Chesebro JH, Fuster V. vWF and cardiovascular disease. *Thromb Haemost.* 1993; 70:111–118. [PubMed: 8236085]
20. Bakker E, Pisteia A, Spaan J, Rolf T, de Vries C, Van Rooijen N, Candi E, VanBavel E. Flow-dependent remodeling of small arteries in mice deficient for tissue-type transglutaminase: possible compensation by macrophage-derived factor XIII. *Circ Res.* 2006; 99:86–92. [PubMed: 16741156]
21. Balakrishnan B, Tzafirri AR, Seifert P, Groothuis A, Rogers C, Edelman ER. Strut position, blood flow, and drug deposition: implications for single and overlapping drug-eluting stents. *Circulation.* 2005; 111:2958–2965. [PubMed: 15927969]
22. Balcells M, Fernandez Suarez M, Vazquez M, Edelman ER. Cells in fluidic environments are sensitive to flow frequency. *J Cell Physiol.* 2005; 204:329–335. [PubMed: 15700266]
23. Balligand JL, Feron O, Dessy C. eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. *Physiol Rev.* 2009; 89:481–534. [PubMed: 19342613]

24. Bao X, Clark CB, Frangos JA. Temporal gradient in shear-induced signaling pathway: involvement of MAP kinase, *c-fos*, and connexin43. *Am J Physiol Heart Circ Physiol*. 2000; 278:H1598–H1605. [PubMed: 10775139]
25. Bao X, Lu C, Frangos JA. Mechanism of temporal gradients in shear-induced ERK1/2 activation and proliferation in endothelial cells. *Am J Physiol Heart Circ Physiol*. 2001; 281:H22–H29. [PubMed: 11406464]
26. Bao X, Lu C, Frangos JA. Temporal gradient in shear but not steady shear stress induces PDGF-A and MCP-1 expression in endothelial cells: role of NO, NF kappa B, egr-1. *Arterioscler Thromb Vasc Biol*. 1999; 19:996–1003. [PubMed: 10195928]
27. Barbee KA, Mundel T, Lal R, Davies PF. Subcellular distribution of shear stress at the surface of flow aligned and non-aligned endothelial monolayers. *Am J Physiol Heart Circ Physiol*. 1995; 268:H1765–H1772.
28. Barber KM, Pinero A, Truskey GA. Effects of recirculating flow on U-937 cell adhesion to human umbilical vein endothelial cells. *Am J Physiol Heart Circ Physiol*. 1998; 275:H591–H599.
29. Bassiouny HS, Song RH, Hong XF, Singh A, Kocharyan H, Glagov S. Flow regulation of 72-kD collagenase IV (MMP-2) after experimental arterial injury. *Circulation*. 1998; 98:157–163. [PubMed: 9679722]
30. Beckman JA. Cardiology patient page. Diseases of the veins. *Circulation*. 2002; 106:2170–2172. [PubMed: 12390942]
31. Beere PA, Glagov S, Zarins CK. Experimental atherosclerosis at the carotid bifurcation of the cynomolgus monkey. Localization, compensatory enlargement, and the sparing effect of lowered heart rate. *Arterioscler Thromb*. 1992; 12:1245–1253. [PubMed: 1420083]
32. Bell FP, Adamson IL, Schwartz CJ. Aortic endothelial permeability to albumin: focal and regional patterns of uptake and transmural distribution of ¹³¹I-albumin in the young pig. *Exp Mol Pathol*. 1974; 20:57–68. [PubMed: 4817736]
33. Beohar N, Davidson CJ, Kip KE, Goodreau L, Vlachos HA, Meyers SN, Benzuly KH, Flaherty JD, Ricciardi MJ, Bennett CL, Williams DO. Outcomes and complications associated with off-label and untested use of drug-eluting stents. *JAMA*. 2007; 297:1992–2000. [PubMed: 17488964]
34. Bergan JJ. Chronic venous insufficiency and the therapeutic effects of Daflon 500 mg. *Angiology*. 2005; 56:S21–S24. [PubMed: 16193222]
35. Bergan JJ, Pascarella L, Schmid-Schönbein GW. Pathogenesis of primary chronic venous disease: insights from animal models of venous hypertension. *J Vasc Surg*. 2008; 47:183–192. [PubMed: 18178472]
36. Bergan JJ, Schmid-Schönbein GW, Smith PD, Nicolaidis AN, Boisseau MR, Eklof B. Chronic venous disease. *N Engl J Med*. 2006; 355:488–498. [PubMed: 16885552]
37. Berk BC. Atheroprotective signaling mechanisms activated by steady laminar flow in endothelial cells. *Circulation*. 2008; 117:1082–1089. [PubMed: 18299513]
38. Berk BC, Abe JI, Min W, Surapisitchat J, Yan C. Endothelial atheroprotective and anti-inflammatory mechanisms. *Ann NY Acad Sci*. 2001; 947:93–111. [PubMed: 11795313]
39. Bharadvaj BK, Mabon RF, Giddens DP. Steady flow in a model of the human carotid bifurcation. Part I. Flow visualization. *J Biomech*. 1982; 15:349–362. [PubMed: 7118950]
40. Blackman BR, Barbee KA, Thibault LE. In vitro cell shearing device to investigate the dynamic response of cells in a controlled hydrodynamic environment. *Ann Biomed Eng*. 2000; 28:363–372. [PubMed: 10870893]
41. Blackman BR, García-Cardena G, Gimbrone MA Jr. A new in vitro model to evaluate differential responses of endothelial cells to simulated arterial shear stress waveforms. *J Biomech Eng*. 2002; 124:397–407. [PubMed: 12188206]
42. Boisseau MR. Roles of mechanical blood forces in vascular diseases. A clinical overview. *Clin Hemorheol Microcirc*. 2005; 33:201–207. [PubMed: 16215286]
43. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol*. 2003; 23:168–175. [PubMed: 12588755]
44. Boo YC, Sorescu G, Boyd N, Shiojima I, Walsh K, Du J, Jo H. Shear stress stimulates phosphorylation of endothelial nitric-oxide synthase at Ser1179 by Akt-independent mechanisms: role of protein kinase. *J Biol Chem*. 2002; 277:3388–3396. [PubMed: 11729190]

45. Boon RA, Horrevoets AJ. Key transcriptional regulators of the vasoprotective effects of shear stress. *Hamostaseologie*. 2009; 29:39–43. [PubMed: 19151844]
46. Bostrom K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest*. 1993; 91:1800–1809. [PubMed: 8473518]
47. Bouskela E, Donyo KA. Effects of oral administration of purified micronized flavonoid fraction on increased microvascular permeability induced by various agents and on ischemia/reperfusion in the hamster cheek pouch. *Angiology*. 1997; 48:391–399. [PubMed: 9158383]
48. Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kaltschmidt C, Baeuerle PA, Neumeier D. Activated transcription factor nuclear factor-kappa B is present in the atherosclerotic lesion. *J Clin Invest*. 1996; 97:1715–1722. [PubMed: 8601637]
49. Breddin HK. Thrombosis and Virchow's triad: what is established? *Semin Thromb Hemost*. 1989; 15:237–239. [PubMed: 2688094]
50. Brooks AR, Lelkes PI, Rubanyi GM. Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow. *Physiol Genomics*. 2002; 9:27–41. [PubMed: 11948288]
51. Brooks AR, Lelkes PI, Rubanyi GM. Gene expression profiling of vascular endothelial cells exposed to fluid mechanical forces: relevance for focal susceptibility to atherosclerosis. *Endothelium*. 2004; 11:45–57. [PubMed: 15203878]
52. Brooks EG, Trotman W, Wadsworth MP, Taatjes DJ, Evans MF, Ittleman FP, Callas PW, Esmon CT, Bovill EG. Valves of the deep venous system: an overlooked risk factor. *Blood*. 2009; 114:1276–1279. [PubMed: 19436051]
53. Brown TD. Techniques for mechanical stimulation of cells in vitro: a review. *J Biomech*. 2000; 33:3–14. [PubMed: 10609513]
54. Bryant M, Drew GM, Houston P, Hissey P, Campbell CJ, Braddock M. Tissue repair with a therapeutic transcription factor. *Hum Gene Ther*. 2000; 11:2143–2158. [PubMed: 11044915]
55. Buchanan JR Jr, Kleinstreuer C, Truskey GA, Lei M. Relation between non-uniform hemodynamics and sites of altered permeability and lesion growth at the rabbit aorto-celiac junction. *Atherosclerosis*. 1990; 143:27–40. [PubMed: 10208478]
56. Buga GM, Gold ME, Fukuto JM, Ignarro LJ. Shear stress-induced release of nitric oxide from endothelial cells grown on beads. *Hypertension*. 1991; 17:187–193. [PubMed: 1991651]
57. Bürrig KF. The endothelium of advanced arteriosclerotic plaques in humans. *Arterioscler Thromb*. 1991; 11:1678–1689. [PubMed: 1657131]
58. Butcher JT, Nerem RM. Valvular endothelial cells and the mechanoregulation of valvular pathology. *Phil Trans R Soc Lond*. 2007; 362:1445–1457. [PubMed: 17569641]
59. Butcher JT, Penrod AM, Garcia AJ, Nerem RM. Unique morphology and focal adhesion development of valvular endothelial cells in static and fluid flow environments. *Arterioscler Thromb Vasc Biol*. 2004; 24:1429–1434. [PubMed: 15117733]
60. Butcher JT, Tressell S, Johnson T, Turner D, Sorescu G, Jo H, Nerem RM. Transcriptional profiles of valvular and vascular endothelial cells reveal phenotypic differences: influence of shear stress. *Arterioscler Thromb Vasc Biol*. 2006; 26:69–77. [PubMed: 16293796]
61. Butler PJ, Norwich G, Weinbaum S, Chien S. Shear stress induces a time- and position-dependent increase in endothelial cell membrane fluidity. *Am J Physiol Cell Physiol*. 2001; 280:C962–C969. [PubMed: 11245613]
62. Butler PJ, Tsou TC, Li JY, Usami S, Chien S. Rate sensitivity of shear-induced changes in the lateral diffusion of endothelial cell membrane lipids: a role for membrane perturbation in shear-induced MAPK activation. *FASEB J*. 2002; 16:216–218. [PubMed: 11744620]
63. Butler PJ, Weinbaum S, Chien S, Lemons DE. Endothelium-dependent, shear-induced vasodilation is rate-sensitive. *Microcirculation*. 2000; 7:53–65. [PubMed: 10708337]
64. Cai H, McNally JS, Weber M, Harrison DG. Oscillatory shear stress upregulation of endothelial nitric oxide synthase requires intracellular hydrogen peroxide and CaMKII. *J Mol Cell Cardiol*. 2004; 37:121–125. [PubMed: 15242742]

65. Carallo C, Irace C, Pujia A, De Franceschi MS, Crescenzo A, Motti C, Cortese C, Mattioli PL, Gnasso A. Evaluation of common carotid hemodynamic forces. Relations with wall thickening. *Hypertension*. 1999; 34:217–221. [PubMed: 10454444]
66. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. *J Pathol*. 2000; 190:255–266. [PubMed: 10685060]
67. Carlier SG, van Damme LC, Blommerde CP, Wentzel JJ, van Langehove G, Verheye S, Kockx MM, Knaapen MW, Cheng C, Gijzen F, Duncker DJ, Stergiopoulos N, Slager CJ, Serruys PW, Krams R. Augmentation of wall shear stress inhibits neointimal hyperplasia after stent implantation: inhibition through reduction of inflammation? *Circulation*. 2003; 107:2741–2746. [PubMed: 12742998]
68. Caro CG, Fitz-Gerald JM, Schroter RC. Arterial wall shear and distribution of early atheroma in man. *Nature*. 1969; 223:1159–1160. [PubMed: 5810692]
69. Caro CG, Fitz-Gerald JM, Schroter RC. Atheroma and arterial wall shear. Observation, correlation and proposal of a shear dependent mass transfer mechanism for atherogenesis. *Proc R Soc Lond B Biol Sci*. 1971; 177:109–159. [PubMed: 4396262]
70. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci USA*. 1995; 92:11190–11194. [PubMed: 7479963]
71. Chang K, Weiss D, Suo J, Vega JD, Giddens D, Taylor WR, Jo H. Bone morphogenic protein antagonists are coexpressed with bone morphogenic protein 4 in endothelial cells exposed to unstable flow in vitro in mouse aortas and in human coronary arteries: role of bone morphogenic protein antagonists in inflammation and atherosclerosis. *Circulation*. 2007; 116:1258–1266. [PubMed: 17785623]
72. Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW. Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium. *Circ Res*. 1998; 82:532–539. [PubMed: 9529157]
73. Charles AK, Gresham GA. Histopathological changes in venous grafts and in varicose and non-varicose veins. *J Clin Pathol*. 1993; 46:603–606. [PubMed: 8157743]
74. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. *J Am Coll Cardiol*. 2007; 49:2379–2393. [PubMed: 17599600]
75. Chen BP, Li YS, Zhao Y, Chen KD, Li S, Lao J, Yuan S, Shyy JY, Chien S. DNA microarray analysis of gene expression in endothelial cells in response to 24-h shear stress. *Physiol Genomics*. 2001; 7:55–63. [PubMed: 11595792]
76. Chen CN, Chang SF, Lee PL, Chang K, Chen LJ, Usami S, Chien S, Chiu JJ. Neutrophils, lymphocytes, and monocytes exhibit diverse behaviors in transendothelial and subendothelial migrations under coculture with smooth muscle cells in disturbed flow. *Blood*. 2006; 107:1933–1942. [PubMed: 16293605]
77. Chen XL, Grey JY, Thomas S, Qiu FH, Medford RM, Wasserman MA, Kunsch C. Sphingosine kinase-1 mediates TNF-alpha-induced MCP-1 gene expression in endothelial cells: upregulation by oscillatory flow. *Am J Physiol Heart Circ Physiol*. 2004; 287:H1452–H1458. [PubMed: 15191888]
78. Chen XL, Varner SE, Rao AS, Grey JY, Thomas S, Cook CK, Wasserman MA, Medford RM, Jaiswal AK, Kunsch C. Laminar flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. *J Biol Chem*. 2003; 278:703–711. [PubMed: 12370194]
79. Chen YL, Jan KM, Lin HS, Chien S. Ultrastructural studies on macromolecular permeability in relation to endothelial cell turnover. *Atherosclerosis*. 1995; 118:89–104. [PubMed: 8579635]
80. Cheng C, de Crom R, van Haperen R, Helderma F, Gourabi BM, van Damme LC, Kirschbaum SW, Slager CJ, van der Steen AF, Krams R. The role of shear stress in atherosclerosis: action through gene expression and inflammation? *Cell Biochem Biophys*. 2004; 41:279–294. [PubMed: 15475614]
81. Cheng C, Tempel D, van Haperen R, de Boer HC, Segers D, Huisman M, van Zonneveld AJ, Leenen PJ, van der Steen A, Serruys PW, de Crom R, Krams R. Shear stress-induced changes in

- atherosclerotic plaque composition are modulated by chemokines. *J Clin Invest.* 2007; 117:616–626. [PubMed: 17304353]
82. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation.* 2006; 113:2744–2753. [PubMed: 16754802]
 83. Cheng C, van Haperen R, de Waard M, van Damme LC, Tempel D, Hanemaaijer L, van Cappellen GW, Bos J, Slager CJ, Duncker DJ, van der Steen AF, de Crom R, Krams R. Shear stress affects the intracellular distribution of eNOS: direct demonstration by a novel in vivo technique. *Blood.* 2005; 106:3691–3698. [PubMed: 16105973]
 84. Chi JT, Chang HY, Haraldsen G, Jahnsen FL, Troyanskaya OG, Chang DS, Wang Z, Rockson SG, van de Rijn M, Botstein D, Brown PO. Endothelial cell diversity revealed by global expression profiling. *Proc Natl Acad Sci USA.* 2003; 100:10623–10628. [PubMed: 12963823]
 85. Chien S. Present state of blood rheology. In: Messmer, K.; Schmid-Schönbein, H., editors. *Hemodilution, Theoretical Basis and Clinical Application.* Basel: Karger; 1972.
 86. Chien S. Molecular and mechanical bases of focal lipid accumulation in arterial wall. *Prog Biophys Mol Biol.* 2003; 83:131–151. [PubMed: 12865076]
 87. Chien S. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *Am J Physiol Heart Circ Physiol.* 2007; 292:H1209–H1224. [PubMed: 17098825]
 88. Chiu JJ, Chen CN, Lee PL, Yang CT, Chuang HS, Chien S, Usami S. Analysis of the effect of disturbed flow on monocytic adhesion to endothelial cells. *J Biomech.* 2003; 36:1883–1895. [PubMed: 14614942]
 89. Chiu JJ, Chen LJ, Chang SF, Lee PL, Lee CI, Tasi MC, Lee DY, Hsieh HP, Usami S, Chien S. Shear stress inhibits smooth muscle cell-induced inflammatory gene expression in endothelial cells: role of NF- κ B. *Arterioscler Thromb Vasc Biol.* 2005; 25:963–969. [PubMed: 15718492]
 90. Chiu JJ, Chen LJ, Lee CI, Lee PL, Tasi MC, Lee DY, Usami S, Chien S. Mechanisms of induction of endothelial cell E-selectin expression by smooth muscle cells and its inhibition by shear stress. *Blood.* 2007; 110:519–528. [PubMed: 17371946]
 91. Chiu JJ, Chen LJ, Lee PL, Lee CI, Lo LW, Usami S, Chien S. Shear stress inhibits adhesion molecule expression in vascular endothelial cells induced by co-culture with smooth muscle cells. *Blood.* 2003; 101:2667–2674. [PubMed: 12468429]
 92. Chiu JJ, Lee PL, Chen CN, Lee CI, Chang SF, Chen LJ, Lien SC, Ko YC, Usami S, Chien S. Shear stress increases ICAM-1 and decreases VCAM-1 and E-selectin expressions induced by tumor necrosis factor- α in endothelial cells. *Arterioscler Thromb Vasc Biol.* 2004; 24:73–79. [PubMed: 14615388]
 93. Chiu JJ, Usami S, Chien S. Vascular endothelial responses to altered shear stress: pathologic implications for atherosclerosis. *Ann Med.* 2009; 41:19–28. [PubMed: 18608132]
 94. Chiu JJ, Wang DL, Chien S, Skalak R, Usami S. Effects of disturbed flow on endothelial cells. *J Biomech Eng.* 1998; 120:2–8. [PubMed: 9675673]
 95. Cho A, Mitchell L, Koopmans D, Langille BL. Effects of changes in blood flow rate on cell death and cell proliferation in carotid arteries of immature rabbits. *Circ Res.* 1997; 81:328–337. [PubMed: 9285634]
 96. Choi SY, Hirata K, Ishida T, Quertermous T, Cooper AD. Endothelial lipase: a new lipase on the block. *J Lipid Res.* 2002; 43:1763–1769. [PubMed: 12401876]
 97. Chuang PT, Cheng HJ, Lin SJ, Jan KM, Lee MM, Chien S. Macromolecular transport across arterial and venous endothelium in rats. Studies with Evans blue-albumin and horseradish peroxidase. *Arteriosclerosis.* 1990; 10:188–197. [PubMed: 2180395]
 98. Clarkson P, Montgomery HE, Mullen MJ, Donald AE, Powe AJ, Bull T, Jubb M, World M, Deanfield JE. Exercise training enhances endothelial function in young men. *J Am Coll Cardiol.* 1999; 33:1379–1385. [PubMed: 10193742]
 99. Clowes AW, Kirkman TR, Clowes MM. Mechanisms of arterial graft failure. II. Chronic endothelial and smooth muscle cell proliferation in healing polytetrafluoroethylene prostheses. *J Vasc Surg.* 1986; 3:877–884. [PubMed: 3712635]
 100. Collins T, Cybulsky MI. NF- κ B: pivotal mediator or innocent bystander in atherogenesis? *J Clin Invest.* 2001; 107:255–264. [PubMed: 11160146]

101. Conklin BS, Zhong DS, Zhao W, Lin PH, Chen C. Shear stress regulates occludin and VEGF expression in porcine arterial endothelial cells. *J Surg Res.* 2002; 102:13–21. [PubMed: 11792146]
102. Cooley BC. Murine model of neointimal formation and stenosis in vein grafts. *Atheroscler Thromb Vasc Biol.* 2004; 24:1180–1185.
103. Cooke JP. Flow, NO and atherogenesis. *Proc Natl Acad Sci USA.* 2003; 100:768–770. [PubMed: 12552094]
104. Corada M, Mariotti M, Thurston G, Smith K, Kunkel R, Brockhaus M, Lampugnani MG, Martin-Padura I, Stoppacciaro A, Roco L, McDonald DM, Ward PA, Dejana E. Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo. *Proc Natl Acad Sci USA.* 1999; 96:9815–9820. [PubMed: 10449777]
105. Cornhill JF, Barrett WA, Herderick EE, Mahley RW, Fry DL. Topographic study of sudanophilic lesions in cholesterol-fed minipigs by image analysis. *Arteriosclerosis.* 1985; 5:415–426. [PubMed: 2412537]
106. Cornhill JF, Herderick EE, Stary HC. Topography of human aortic sudanophilic lesions. *Monogr Atheroscler.* 1990; 15:13–19. [PubMed: 2296239]
107. Cornhill JF, Levesque MJ, Herderick EE, Nerem RM, Kilman JW, Vasko JS. Quantitative study of the rabbit aortic endothelium using vascular casts. *Atherosclerosis.* 1980; 35:321–337. [PubMed: 7362703]
108. Cornhill JF, Roach MR. A quantitative study of the localization of atherosclerotic lesions in the rabbit aorta. *Atherosclerosis.* 1976; 23:489–501. [PubMed: 57782]
109. Corti R, Fuster V, Badimon JJ, Hutter R, Fayad ZA. New understanding of atherosclerosis (clinically and experimentally) with evolving MRI technology in vivo. *Ann NY Acad Sci.* 2001; 947:181–198. [PubMed: 11795266]
110. Cuff CA, Kothapalli D, Azonobi I, Chun S, Zhang Y, Belkin R, Yeh C, Secreto A, Assoian RK, Rader DJ, Puré E. The adhesion receptor CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. *J Clin Invest.* 2001; 108:1031–1040. [PubMed: 11581304]
111. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. *Lab Invest.* 2005; 85:9–23. [PubMed: 15568038]
112. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest.* 2001; 107:1255–1262. [PubMed: 11375415]
113. Da Silva AF, Carpenter T, How TV, Harris PL. Stable vortices within vein cuffs inhibit anastomotic myointimal hyperplasia? *Eur J Vasc Endovasc Surg.* 1997; 14:157–163. [PubMed: 9345233]
114. Da Silva RF, Chambaz C, Stergiopoulos N, Hayoz D, Silacci P. Transcriptional and post-transcriptional regulation of preproendothelin-1 by plaque-prone hemodynamics. *Atherosclerosis.* 2007; 194:383–390. [PubMed: 17324434]
115. Dai G, Kaazempur-Mofrad MR, Natarajan S, Zhang Y, Vaughn S, Blackman BR, Kamm RD, García-Cardena G, Gimbrone MA Jr. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and-resistant regions of human vasculature. *Proc Natl Acad Sci USA.* 2004; 101:14871–14876. [PubMed: 15466704]
116. Dai G, Vaughn S, Zhang Y, Wang ET, Garcia-Cardena G, Gimbrone MA Jr. Biomechanical forces in atherosclerosis-resistant vascular regions regulate endothelial redox balance via phosphoinositol 3-kinase/Akt-dependent activation of Nrf2. *Circ Res.* 2007; 101:723–733. [PubMed: 17673673]
117. Dalager S, Paaske WP, Kristensen IB, Laurberg JM, Falk E. Artery-related differences in atherosclerosis expression: implications for atherogenesis and dynamics in intima-media thickness. *Stroke.* 2007; 38:2698–2705. [PubMed: 17761918]
118. Dancu MB, Tarbell JM. Coronary endothelium expresses a pathologic gene pattern compared to aortic endothelium: correlation of asynchronous hemodynamics and pathology in vivo. *Atherosclerosis.* 2007; 192:9–14. [PubMed: 16806232]

119. Dardik A, Chen L, Frattini J, Asada H, Aziz F, Kudo FA, Sumpio BE. Differential effects of orbital and laminar shear stress on endothelial cells. *J Vasc Surg.* 2005; 41:869–880. [PubMed: 15886673]
120. Dardik H, Silvestri F, Alasio T, Berry S, Kahn M, Ibrahim IM, Sussman B, Wolodiger F. Improved method to create the common ostium variant of the distal arteriovenous fistula for enhancing crural prosthetic graft patency. *J Vasc Surg.* 1996; 24:240–248. [PubMed: 8752035]
121. Dardik H, Sussman B, Ibrahim IM, Kahn M, Svoboda JJ, Mendes D, Dardik I. Distal arteriovenous fistula as an adjunct to maintaining arterial and graft patency for limb salvage. *Surgery.* 1983; 94:478–486. [PubMed: 6612582]
122. Dasi LP, Sucosky P, de Zelicourt D, Sundareswaran K, Jimenez J, Yoganathan AP. Advances in cardiovascular fluid mechanics: bench to bedside. *Ann NY Acad Sci.* 2009; 1161:1–25. [PubMed: 19426302]
123. Davies MG, Hagen PO. Pathophysiology of vein graft failure: a review. *Eur J Vasc Endovasc Surg.* 1995; 9:7–18. [PubMed: 7664016]
124. Davies MG, Huynh TT, Fulton GJ, Barber L, Svendsen E, Hagen PO. Early morphology of accelerated vein graft atheroma in experimental vein grafts. *Ann Vasc Surg.* 1999; 13:378–385. [PubMed: 10398734]
125. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev.* 1995; 75:519–560. [PubMed: 7624393]
126. Davies PF. Spatial hemodynamics, the endothelium, and focal atherogenesis: A cell cycle link? *Circ Res.* 2000; 86:114–116. [PubMed: 10666402]
127. Davies PF. Endothelial mechanisms of flow-mediated athero-protection and susceptibility. *Circ Res.* 2007; 101:10–12. [PubMed: 17615377]
128. Davies PF, Barbee KA, Volin MV, Robotewskyj A, Chen J, Joseph L, Griem ML, Wernick MN, Jacobs E, Polacek DC, dePaola N, Barakat AI. Spatial relationships in early signaling events of flow-mediated endothelial mechanotransduction. *Annu Rev Physiol.* 1997; 59:527–549. [PubMed: 9074776]
129. Davies, PF.; Helmke, BP. Endothelial mechanotransduction. In: Mofrad, MRK.; Kamm, RD., editors. *Cellular Mechanotransduction.* London: Cambridge Univ. Press; 2009.
130. Davies PF, Polacek DC, Handen JS, Helmke BP, DePaola N. A spatial approach to transcriptional profiling: mechanotransduction and the focal origin of atherosclerosis. *Trends Biotechnol.* 1999; 17:347–335. [PubMed: 10461179]
131. Davies PF, Polacek DC, Shi C, Helmke BP. The convergence of haemodynamics, genomics, and endothelial structure in studies of the focal origin of atherosclerosis. *Biorheology.* 2002; 39:299–306. [PubMed: 12122245]
132. Davies P, Remuzzi A, Gordon E, Dewey C, Gimbrone M. Turbulent fluid shear stress induces vascular endothelial cell turnover in vitro. *Proc Natl Acad Sci USA.* 1986; 83:2114–2117. [PubMed: 3457378]
133. Davies PF, Robotewskyj A, Griem ML. Quantitative studies of endothelial cell adhesion. Directional remodeling of focal adhesion sites in response to flow forces. *J Clin Invest.* 1994; 93:2031–2038. [PubMed: 8182135]
134. Davies PF, Shi C, DePaola N, Helmke BP, Polacek DC. Hemodynamics and the focal origin of atherosclerosis: a spatial approach to endothelial structure, gene expression, and function. *Ann NY Acad Sci.* 2001; 947:7–17. [PubMed: 11795312]
135. Davies PF, Spaan JA, Krams R. Shear stress biology of the endothelium. *Ann Biomed Eng.* 2005; 33:1714–1718. [PubMed: 16389518]
136. Davis ME, Cai H, Drummond GR, Harrison DG. Shear stress regulates endothelial nitric oxide synthase expression through c-*Src* by divergent signaling pathways. *Circ Res.* 2001; 89:1073–1080. [PubMed: 11717166]
137. Day SM, Reeve JL, Pedersen B, Farris DM, Myers DD, Im M, Wakefield TW, Mackman N, Fay WP. Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood.* 2005; 105:192–198. [PubMed: 15339841]
138. De Keulenaer GW, Chappell DC, Ishizaka N, Nerem RM, Alexander RW, Griendling KK. Oscillatory and steady laminar shear stress differentially affect human endothelial redox state:

- role of a superoxide-producing NADH oxidase. *Circ Res.* 1998; 82:1094–1101. [PubMed: 9622162]
139. De Nigris F, Lerman LO, Condorelli M, Lerman A, Napoli C. Oxidation-sensitive transcription factors and molecular mechanisms in the arterial wall. *Antioxid Redox Signal.* 2001; 3:1119–1130. [PubMed: 11813985]
140. De Nigris F, Lerman LO, Ignarro SW, Sica G, Lerman A, Palinski W, Ignarro LJ, Napoli C. Beneficial effects of antioxidants and L-arginine on oxidation-sensitive gene expression and endothelial NO synthase activity at sites of disturbed shear stress. *Proc Natl Acad Sci USA.* 2003; 100:1420–1425. [PubMed: 12525696]
141. Deatrick KB, Eliason JL, Lynch EM, Moore AJ, Dewyer NA, Varma MR, Pearce CG, Upchurch GR, Wakefield TW, Henke PK. Vein wall remodeling after deep vein thrombosis involves matrix metalloproteinases and late fibrosis in a mouse model. *J Vasc Surg.* 2005; 42:140–148. [PubMed: 16012463]
142. Dejana E. Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis. *J Clin Invest.* 1996; 98:1949–1953. [PubMed: 8903311]
143. Dekker RJ, Boon RA, Rondaij MG, Kragt A, Volger OL, Elderkamp YW, Meijers JC, Voorberg J, Pannekoek H, Horrevoets AJ. KLF2 provokes a gene expression pattern that establishes functional quiescent differentiation of the endothelium. *Blood.* 2006; 107:4354–4363. [PubMed: 16455954]
144. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, Pannekoek H, Horrevoets AJ. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). *Blood.* 2002; 100:1689–1698. [PubMed: 12176889]
145. Dekker RJ, van Thienen JV, Rohlena J, de Jager SC, Elderkamp YW, Seppen J, de Vries CJ, Biessen EA, van Berkel TJ, Pannekoek H, Horrevoets AJ. Endothelial KLF2 links local arterial shear stress levels to the expression of vascular tone-regulating genes. *Am J Pathol.* 2005; 167:609–618. [PubMed: 16049344]
146. Deng DX, Tsalenko A, Vailaya A, Ben-Dor A, Kundu R, Estay I, Tabibiazar R, Kincaid R, Yakhini Z, Bruhn L, Quertermous T. Differences in vascular bed disease susceptibility reflect differences in gene expression response to atherogenic stimuli. *Circ Res.* 2006; 98:200–208. [PubMed: 16373601]
147. DePaola N, Davies PF, Pritchard WF, Florez L, Harbeck N, Polacek DC. Spatial and temporal regulation of gap junction connexin43 in vascular endothelial cells exposed to controlled disturbed flows in vitro. *Proc Natl Acad Sci USA.* 1999; 96:3154–3159. [PubMed: 10077653]
148. DePaola N, Gimbrone MA Jr, Davies PF, Dewey CF. Vascular endothelium responds to fluid shear stress gradients. *Arterioscler Thromb.* 1992; 12:1254–1257. [PubMed: 1420084]
149. Dewey CF Jr, Bussolari SR, Gimbrone MA Jr, Davies PF. The dynamic response of vascular endothelial cells to fluid shear stress. *J Biomech Eng.* 1981; 103:177–185. [PubMed: 7278196]
150. Dewey CF Jr. Effects of fluid flow on living vascular cells. *J Biomech Eng.* 1984; 106:31–35. [PubMed: 6539406]
151. Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, Tordoir JH, Spronk HM, Vermeer C, Daemen MJ. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. *Arterioscler Thromb Vasc Biol.* 2001; 21:1998–2003. [PubMed: 11742876]
152. Di Stefano I, Koopmans DR, Langille BL. Modulation of arterial growth of the rabbit carotid artery associated with experimental elevation of blood flow. *J Vasc Res.* 1998; 35:1–7. [PubMed: 9482690]
153. Diamond SL, Eskin SG, McIntire LV. Fluid flow stimulates tissue plasminogen activator secretion by cultured human endothelial cells. *Science.* 1989; 243:1483–1485. [PubMed: 2467379]
154. Dimmeler S, Hermann C, Galle J, Zeiher AM. Upregulation of superoxide dismutase and nitric oxide synthase mediates the apoptosis- suppressive effects of shear stress on endothelial cells. *Arterioscler Thromb Vasc Biol.* 1999; 19:656–664. [PubMed: 10073970]

155. Dimmeler S, Haendeler J, Rippmann V, Nehls M, Zeiher A. Shear stress inhibits apoptosis of human endothelial cells. *FEBS Lett.* 1996; 399:71–74. [PubMed: 8980122]
156. Dimmeler S, Haendeler J, Zeiher AM. Regulation of endothelial cell apoptosis in atherothrombosis. *Curr Opin Lipidol.* 2002; 13:531–536. [PubMed: 12352017]
157. Downing LJ, Strieter RM, Kadell AM, Wilke CA, Brown SL, Wroblewski SK, Burdick MD, Hulin MS, Fowlkes JB, Greenfield LJ, Wakefield TW. Neutrophils are the initial cell type identified in deep venous thrombosis induced vein wall inflammation. *ASAIO J.* 1996; 42:M677–M682. [PubMed: 8944966]
158. Downing LJ, Wakefield TW, Strieter RM, Prince MR, Londy FJ, Fowlkes JB, Hulin MS, Kadell AM, Wilke CA, Brown SL, Wroblewski SK, Burdick MD, Anderson DC, Greenfield LJ. Anti-P-selectin antibody decreases inflammation and thrombus formation in venous thrombosis. *J Vasc Surg.* 1997; 25:816–827. [PubMed: 9152309]
159. Drexler H, Hornig B. Endothelial dysfunction in human disease. *J Mol Cell Cardiol.* 1999; 31:51–60. [PubMed: 10072715]
160. Duncker DJ, Bache RJ. Regulation of coronary blood flow during exercise. *Physiol Rev.* 2008; 88:1009–1086. [PubMed: 18626066]
161. Dunzendorfer S, Lee HK, Tobias PS. Flow-dependent regulation of endothelial Toll-like receptor 2 expression through inhibition of SP1 activity. *Circ Res.* 2004; 95:684–691. [PubMed: 15345653]
162. Duraiswamy N, Schoepfoerster RT, Moreno MR, Moore JE Jr. Stented artery flow patterns and their effects on the artery wall. *Annu Rev Fluid Mech.* 2006; 39:357–382.
163. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. *Circulation.* 2005; 111:2398–2440. [PubMed: 15883226]
164. Edelman ER, Rogers C. Pathobiologic responses to stenting. *Am J Cardiol.* 1998; 81:4E–6E.
165. Edelman ER, Rogers C. Stent-versus-stent equivalency trials: are some stents more equal than others? *Circulation.* 1999; 100:896–898. [PubMed: 10468517]
166. Edfeldt K, Swedenborg J, Hansson GK, Yan Z. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. *Circulation.* 2002; 105:1158–1161. [PubMed: 11889007]
167. El-Omar M, Dangas G, Iakovou I, Mehran R. Update on in-stent restenosis. *Curr Interv Cardiol Rep.* 2001; 3:296–305. [PubMed: 11696295]
168. Endres M, Laufs U, Merz H, Kaps M. Focal expression of intercellular adhesion molecule-1 in the human carotid bifurcation. *Stroke.* 1997; 28:77–82. [PubMed: 8996493]
169. Eppihimer MJ, Schaub RG. P-Selectin-dependent inhibition of thrombosis during venous stasis. *Arterioscler Thromb Vasc Biol.* 2000; 20:2483–2488. [PubMed: 11073856]
170. Erami C, Zhang H, Tanoue A, Tsujimoto G, Thomas S, Faber J. Adrenergic catecholamine trophic activity contributes to flow-mediated arterial remodeling. *Am J Physiol Heart Circ Physiol.* 2005; 289:H744–H753. [PubMed: 15849236]
171. Eriksson EE, Karlof E, Lundmark K, Rotzius P, Hedin U, Xie X. Powerful inflammatory properties of large vein endothelium in vivo. *Arterioscler Thromb Vasc Biol.* 2005; 25:723–728. [PubMed: 15681299]
172. Eskin SG, Ives CL, McIntire LV, Navarro LT. Response of cultured endothelial cells to steady flow. *Microvasc Res.* 1984; 28:87–94. [PubMed: 6748961]
173. Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, Celi A, Croce K, Furie BC, Furie B. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J Exp Med.* 2003; 197:1585–1598. [PubMed: 12782720]
174. Félétou M, Vanhoutte PM. Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol.* 2006; 26:1215–1225. [PubMed: 16543495]
175. Félétou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol.* 2006; 291:H985–H1002. [PubMed: 16632549]
176. Finn AV, Joner M, Nakazawa G, Kolodgie F, Newell J, John MC, Gold HK, Virmani R. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. *Circulation.* 2007; 115:2435–2441. [PubMed: 17438147]

177. Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, Gold HK, Virmani R. Vascular responses to drug eluting stents: importance of delayed healing. *Arterioscler Thromb Vasc Biol.* 2007; 27:1500–1510. [PubMed: 17510464]
178. Fisher RK, How TV, Toonder IM, Hoedt MT, Brennan JA, Gilling-Smith GL, Harris PL. Harnessing haemodynamic forces for the suppression of anastomotic intimal hyperplasia: the rationale for precuffed grafts. *Eur J Vasc Endovasc Surg.* 2001; 21:520–528. [PubMed: 11397026]
179. Fledderus JO, Boon RA, Volger OL, Hurttala H, Ylä-Herttuala S, Pannekoek H, Levonen AL, Horrevoets AJ. KLF2 primes the antioxidant transcription factor Nrf2 for activation in endothelial cells. *Arterioscler Thromb Vasc Biol.* 2008; 28:1339–1346. [PubMed: 18467642]
180. Fledderus JO, van Thienen JV, Boon RA, Dekker RJ, Rohlena J, Volger OL, Bijmens AP, Daemen MJ, Kuiper J, van Berkel TJ, Pannekoek H, Horrevoets AJ. Prolonged shear stress and KLF2 suppress constitutive proinflammatory transcription through inhibition of ATF2. *Blood.* 2007; 109:4249–4257. [PubMed: 17244683]
181. Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circ Res.* 2003; 93:e136–e142. [PubMed: 14563712]
182. Forman MB, Virmani R, Puett DW. Mechanisms and therapy of myocardial reperfusion injury. *Circulation.* 1990; 81:IV69–IV78. [PubMed: 2407376]
183. Fox B, James K, Morgan B, Seed A. Distribution of fatty and fibrous plaques in young human coronary arteries. *Atherosclerosis.* 1982; 41:337–347. [PubMed: 7066081]
184. Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production by cultured human endothelial cells. *Science.* 1985; 227:1477–1479. [PubMed: 3883488]
185. Frangos JA, McIntire LV, Eskin SG. Shear stress induced stimulation of mammalian cell metabolism. *Biotechnol Bioeng.* 1988; 32:1053–1060. [PubMed: 18587822]
186. Frangos SG, Gahtan V, Sumpio B. Localization of atherosclerosis: role of hemodynamics. *Arch Surg.* 1999; 134:1142–1149. [PubMed: 10522862]
187. Franke RP, Graefe M, Schnittler H, Seiffge D, Mittermayer C, Drenckhahn D. Induction of human vascular endothelial stress fibres by fluid shear stress. *Nature.* 1984; 307:648–649. [PubMed: 6537993]
188. Friedman MH, Barger CB, Deters OJ, Hutchins GM, Mark FF. Correlation between wall shear and intimal thickness at a coronary artery branch. *Atherosclerosis.* 1987; 68:27–33. [PubMed: 3689481]
189. Friedman MH, Brinkman AM, Qin JJ, Seed WA. Relation between coronary artery geometry and the distribution of early sudanophilic lesions. *Atherosclerosis.* 1993; 98:193–199. [PubMed: 7681291]
190. Friedman MH, Hutchins GM, Bergerson CB, Deters OJ, Mark FF. Correlation between intimal thickness and fluid shear in human arteries. *Atherosclerosis.* 1981; 39:425–436. [PubMed: 7259822]
191. Friesenecker B, Tsai AG, Allegra C, Intaglietta M. Oral administration of purified micronized flavonoid fraction suppresses leukocyte adhesion in ischemia-reperfusion injury: in vivo observations in the hamster skin fold. *Int J Microcirc Clin Exp.* 1994; 14:50–55. [PubMed: 7960444]
192. Fung, YC. *Biomechanics: Circulation.* New York: Springer; 1997.
193. Funk SD, Yurdagul A Jr, Green JM, Jhaveri KA, Schwartz MA, Orr AW. Matrix-specific protein kinase A signaling regulates p21-activated kinase activation by flow in endothelial cells. *Circ Res.* 2010; 106:1394–1403. [PubMed: 20224042]
194. Furuse A, Klopp EH, Brawley RK, Bott VL. Hemodynamics of aorta-to-coronary artery bypass. *Ann Thorac Surg.* 1972; 14:282–293. [PubMed: 4538214]
195. Gabriels JE, Paul DL. Connexin43 is highly localized to sites of disturbed flow in rat aortic endothelium but connexin37 and connexin40 are more uniformly distributed. *Circ Res.* 1998; 83:636–643. [PubMed: 9742059]
196. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, the ugly. *Circ Res.* 2002; 90:251–262. [PubMed: 11861412]

197. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007; 27:2292–2301. [PubMed: 17673705]
198. Gallik S, Usami S, Jan KM, Chien S. Shear stress-induced detachment of human polymorphonuclear leukocytes from endothelial cell monolayers. *Biorheology.* 1989; 26:823–834. [PubMed: 2611374]
199. Gambillara V, Chambaz C, Roy S, Stergiopulos N, Silacci P. Plaque-prone hemodynamics impairs endothelial function in pig carotid arteries. *Am J Physiol Heart Circ Physiol.* 2006; 290:H2320–H2328. [PubMed: 16415081]
200. Gambillara V, Montorzi G, Haziza-Pigeon C, Stergiopulos N, Silacci P. Arterial wall response to ex vivo exposure to oscillatory shear stress. *J Vasc Res.* 2005; 42:535–544. [PubMed: 16179795]
201. Garcia-Cardena G, Comander J, Anderson K, Blackman B, Gimbrone M. Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. *Proc Natl Acad Sci USA.* 2001; 98:4478–4485. [PubMed: 11296290]
202. Garin G, Abe JI, Mohan A, Lu W, Yan C, Newby AC, Rhaman A, Berk BC. Flow antagonizes TNF-alpha signaling in endothelial cells by inhibiting caspase-dependent PKC-zeta processing. *Circ Res.* 2007; 101:97–105. [PubMed: 17525369]
203. Garin G, Berk BC. Flow-mediated signaling modulates endothelial cell phenotype. *Endothelium.* 2006; 13:375–384. [PubMed: 17169770]
204. Gautam M, Shen Y, Thirkill TL, Douglas GC, Barakat AI. Flow-activated chloride channels in vascular endothelium. Shear stress sensitivity, desensitization dynamics, and physiological implications. *J Biol Chem.* 2006; 281:36492–36500. [PubMed: 16973617]
205. Gerrity GR, Natarajan R, Nadler JL, Kimsey T. Diabetes-induced accelerated atherosclerosis in swine. *Diabetes.* 2001; 50:1654–1665. [PubMed: 11423488]
206. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N Engl J Med.* 1994; 330:1431–1438. [PubMed: 8159199]
207. Gibson CM, Diaz L, Kandarpa K, Sacks FM, Pasternak RC, Sandor T, Feldman C, Stone PH. Relation of vessel wall shear stress to atherosclerosis progression in human coronary arteries. *Arterioscler Thromb.* 1993; 13:310–315. [PubMed: 8427866]
208. Giddens DP, Zarins CK, Glagov S. The role of fluid mechanics in the localization and detection of atherosclerosis. *J Biomech Eng.* 1993; 115:588–594. [PubMed: 8302046]
209. Gimbrone MA Jr. Vascular endothelium: an integrator of pathophysiological stimuli in atherosclerosis. *Am J Cardiol.* 1995; 75:67B–70B.
210. Gimbrone MA Jr. Vascular endothelium, hemodynamic forces, and atherogenesis. *Am J Pathol.* 1999; 155:1–5. [PubMed: 10393828]
211. Gimbrone MA Jr, Anderson KR, Topper JN, Langille BL, Clowes AW, Berce S, Davies MG, Stenmark KR, Frid MG, Weiser-Evans MC, Aldashev AA, Nemenoff RA, Majesky MW, Landerholm TE, Lu J, Ito WD, Arras M, Scholz D, Imhof B, Aurrand-Lions M, Schaper W, Nagel TE, Resnick N, Dewey CF, Gimbrone MA, Davies PF. The critical role of mechanical forces in blood vessel development, physiology and pathology. *J Vasc Surg.* 1999; 29:1104–1151. [PubMed: 10359945]
212. Gimbrone MA Jr, Resnick N, Nagel T, Khachigian LM, Collins T, Topper JN. Hemodynamics, endothelial gene expression, and atherogenesis. *Ann NY Acad Sci.* 1997; 811:1–11. [PubMed: 9186579]
213. Gimbrone MA Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Ann NY Acad Sci.* 2000; 902:230–240. [PubMed: 10865843]
214. Girerd X, London G, Boutouyrie P, Mourad JJ, Safar M, Laurent S. Remodeling of the radial artery in response to a chronic increase in shear stress. *Hypertension.* 1996; 27:799–803. [PubMed: 8613243]
215. Glagov S, Rowley DA, Kohut RI. Atherosclerosis of human aorta and its coronary and renal arteries: a consideration of some hemodynamic factors which may be related to the marked differences in atherosclerotic involvement of the coronary and renal arteries. *Arch Pathol.* 1961; 72:558–571. [PubMed: 13899082]

216. Glagov S, Zarins C, Giddens DP, Ku DN. Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries. *Arch Pathol Lab Med.* 1988; 112:1018–1031. [PubMed: 3052352]
217. Gnasso A, Irace C, Carallo C, De Franceschi MS, Motti C, Mattioli PL, Pujia A. In vivo association between low wall shear stress and plaque in subjects with asymmetrical carotid atherosclerosis. *Stroke.* 1997; 28:993–998. [PubMed: 9158640]
218. Godin D, Ivan E, Johnson C, Magid R, Galis ZS. Remodeling of carotid artery is associated with increased expression of matrix metalloproteinases in mouse blood flow cessation model. *Circulation.* 2000; 102:2861–2866. [PubMed: 11104745]
219. Goel R, Schrank BR, Arora S, Boylan B, Fleming B, Miura H, Newman PJ, Molthen RC, Newman DK. Site-specific effects of PECAM-1 on atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol.* 2008; 28:1996–2002. [PubMed: 18669884]
220. Gonzalez-Navarro H, Burks DJ, Andres V. Murine models to investigate the influence of diabetic metabolism on the development of atherosclerosis and restenosis. *Front Biosci.* 2007; 12:4439–4455. [PubMed: 17485387]
221. Gouverneur M, Spaan JA, Pannekoek H, Fontijn RD, Vink H. Fluid shear stress stimulates incorporation of hyaluronan into endothelial cell glycocalyx. *Am J Physiol Heart Circ Physiol.* 2006; 290:H452–H458.
222. Gradman WS, Segalowitz J, Grundfest W. Venoscopy in varicose vein surgery: initial experience. *Phlebology.* 2003; 8:145–150.
223. Granger DN. Ischemia-reperfusion: mechanisms of microvascular dysfunction and the influence of risk factors for cardiovascular disease. *Microcirculation.* 1999; 6:167–178. [PubMed: 10501090]
224. Greve JM, Les AS, Tang BT, Draney Blomme MT, Wilson NM, Dalman RL, Pelc NJ, Taylor CA. Allometric scaling of wall shear stress from mice to humans: quantification using cine phase-contrast MRI and computational fluid dynamics. *Am J Physiol Heart Circ Physiol.* 2006; 291:H1700–H1708. [PubMed: 16714362]
225. Groenendijk BC, Hierck BP, Gittenberger-de Groot AC, Poelmann RE. Development-related changes in the expression of shear stress responsive genes KLF-2, ET-1, and NOS-3 in the developing cardiovascular system of chicken embryos. *Dev Dyn.* 2004; 230:57–68. [PubMed: 15108309]
226. Groenendijk BC, Hierck BP, Vrolijk J, Baiker M, Pourquie MJ, Gittenberger-de Groot AC, Poelmann RE. Changes in shear stress-related gene expression after experimentally altered venous return in the chicken embryo. *Circ Res.* 2005; 96:1291–1298. [PubMed: 15920020]
227. Gudi S, Nolan JP, Frangos JA. Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition. *Proc Natl Acad Sci USA.* 1998; 95:2515–2519. [PubMed: 9482917]
228. Guo D, Chien S, Shyy JY. Regulation of endothelial cell cycle by laminar versus oscillatory flow: distinct modes of interactions of AMP-activated protein kinase and Akt pathways. *Circ Res.* 2007; 100:564–571. [PubMed: 17272808]
229. Hahn C, Orr AW, Sanders JM, Jhaveri KA, Schwartz MA. The subendothelial extracellular matrix modulates JNK activation by flow. *Circ Res.* 2009; 104:995–1003. [PubMed: 19286608]
230. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol.* 2009; 10:53–62. [PubMed: 19197332]
231. Hahn TL, Unthank JL, Lalka SG. Increased hindlimb leukocyte concentration in a chronic rodent model of venous hypertension. *J Surg Res.* 1999; 81:38–41. [PubMed: 9889055]
232. Hahn TL, Whitfield R, Salter J, Granger DN, Unthank JL, Lalka SG. Evaluation of the role of intercellular adhesion molecule 1 in a rodent model of chronic venous hypertension. *J Surg Res.* 2000; 88:150–154. [PubMed: 10644481]
233. Haidekker MA, L'Heureux N, Frangos JA. Fluid shear stress increases membrane fluidity in endothelial cells: a study with DCVJ fluorescence. *Am J Physiol Heart Circ Physiol.* 2000; 278:H1401–H1406. [PubMed: 10749738]
234. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. The NF- κ B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to

- atherosclerotic lesion formation. *Proc Natl Acad Sci USA*. 2000; 97:9052–9057. [PubMed: 10922059]
235. Hamer JD, Malone PC, Silver IA. The PO₂ in venous valve pockets: its possible bearing on thrombogenesis. *Br J Surg*. 1981; 68:166–170. [PubMed: 7470818]
236. Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest*. 1997; 100:2153–2157. [PubMed: 9410891]
237. Harrison DG. The shear stress of keeping arteries clear. *Nat Med*. 2005; 11:375–376. [PubMed: 15812513]
238. Harrison DG, Widder J, Grumbach I, Chen W, Weber M, Searles C. Endothelial mechanotransduction, nitric oxide and vascular inflammation. *J Intern Med*. 2006; 259:351–363. [PubMed: 16594903]
239. Harry BL, Sanders JM, Feaver RE, Lansley M, Deem TL, Zarbock A, Bruce AC, Pryor AW, Gelfand BD, Blackman BR, Schwartz MA, Ley K. Endothelial cell PECAM-1 promotes atherosclerotic lesions in areas of disturbed flow in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol*. 2008; 28:2003–2008. [PubMed: 18688018]
240. Hastings NE, Simmers MB, McDonald OG, Wamhoff BR, Blackman BR. Atherosclerosis-prone hemodynamics differentially regulates endothelial and smooth muscle cell phenotypes and promotes pro-inflammatory priming. *Am J Physiol Cell Physiol*. 2007; 6:C1824–C1833. [PubMed: 17913848]
241. Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res*. 2004; 95:449–458. [PubMed: 15345667]
242. Helderman F, Segers D, de Crom R, Hierck BP, Poelmann RE, Evans PC, Krams R. Effect of shear stress on vascular inflammation and plaque development. *Curr Opin Lipidol*. 2007; 18:527–533. [PubMed: 17885423]
243. Helmke BP, Goldman RD, Davies PF. Rapid displacement of vimentin intermediate filaments in living endothelial cells exposed to flow. *Circ Res*. 2000; 86:745–752. [PubMed: 10764407]
244. Helmlinger G, Berk BC, Nerem RM. Calcium responses of endothelial cell monolayers subjected to pulsatile, and steady laminar flow differ. *Am J Physiol Cell Physiol*. 1995; 269:C367–C375.
245. Helmlinger G, Geiger RV, Schreck S, Nerem RM. Effects of pulsatile flow on cultured vascular endothelial cell morphology. *J Biomech Eng*. 1991; 113:123–131. [PubMed: 1875686]
246. Henke PK, Pearce CG, Moaveni DM, Moore AJ, Lynch EM, Longo C, Varma M, Dewyer NA, Deatrck KB, Upchurch GR Jr, Wakefield TW, Hogaboam C, Kunkel SL. Targeted deletion of CCR2 impairs deep vein thrombosis resolution in a mouse model. *J Immunol*. 2006; 177:3388–3397. [PubMed: 16920980]
247. Henke PK, Varga A, De S, Deatrck CB, Eliason J, Arenberg DA, Sukheepod P, Thanaporn P, Kunkel SL, Upchurch GR Jr, Wakefield TW. Deep vein thrombosis resolution is modulated by monocyte CXCR2-mediated activity in a mouse model. *Arterioscler Thromb Vasc Biol*. 2004; 24:1130–1137. [PubMed: 15105284]
248. Herbert JM, Bernat A, Maffrand JP. Importance of platelets in experimental venous thrombosis in the rat. *Blood*. 1992; 80:2281–2286. [PubMed: 1421399]
249. Hermann C, Zeiher AM, Dimmeler S. Shear stress inhibits H₂O₂-induced apoptosis of human endothelial cells by modulation of the glutathione redox cycle and nitric oxide synthase. *Arterioscler Thromb Vasc Biol*. 1997; 17:3588–3592. [PubMed: 9437209]
250. Himburg HA, Grzybowski DM, Hazel AL, LaMack JA, Li XM, Friedman MH. Spatial comparison between wall shear stress measures and porcine arterial endothelial permeability. *Am J Physiol Heart Circ Physiol*. 2004; 286:H1916–H1922. [PubMed: 14715506]
251. Hippenstiel S, Suttorp N. Interaction of pathogens with the endothelium. *Thromb Haemost*. 2003; 89:18–24. [PubMed: 12540949]
252. Hochleitner BW, Hochleitner EO, Obrist P, Eberl T, Amberger A, Xu Q, Margreiter R, Wick G. Fluid shear stress induces heat shock protein 60 expression in endothelial cells in vitro and in vivo. *Arterioscler Thromb Vasc Biol*. 2000; 20:617–623. [PubMed: 10712382]
253. Hoffmann B, Moebus S, Möhlenkamp S, Stang A, Lehmann N, Dragano N, Schmermund A, Memmesheimer M, Mann K, Erbel R, Jöckel KH. Heinz Nixdorf Recall Study Investigative

- Group. Residential exposure to traffic is associated with coronary atherosclerosis. *Circulation*. 2007; 116:489–496. [PubMed: 17638927]
254. Hosoya T, Maruyama A, Kang MI, Kawatani Y, Shibata T, Uchida K, Warabi E, Noguchi N, Itoh K, Yamamoto M. Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells. *J Biol Chem*. 2005; 280:27244–27250. [PubMed: 15917255]
255. Houston P, White BP, Campbell CJ, Braddock M. Delivery and expression of fluid shear stress-inducible promoters to the vessel wall: applications for cardiovascular gene therapy. *Hum Gene Ther*. 1999; 10:3031–3044. [PubMed: 10609662]
256. Hsiai TK, Cho SK, Reddy S, Hama S, Navab M, Demer LL, Honda HM, Ho CM. Pulsatile flow regulates monocyte adhesion to oxidized lipid-induced endothelial cells. *Arterioscler Thromb Vasc Biol*. 2001; 21:1770–1776. [PubMed: 11701464]
257. Hsiai TK, Cho SK, Wong PK, Ing M, Salazar A, Sevanian A, Navab M, Demer LL, Ho CM. Monocyte recruitment to endothelial cells in response to oscillatory shear stress. *FASEB J*. 2003; 17:1648–1657. [PubMed: 12958171]
258. Hsiai TK, Hwang J, Barr ML, Correa A, Hamilton R, Alavi M, Rouhanizadeh M, Cadenas E, Hazen SL. Hemodynamics influences vascular peroxynitrite formation: implication for low-density lipoprotein apo-B-100 nitration. *Free Radic Biol Med*. 2007; 42:519–529. [PubMed: 17275684]
259. Hsu PP, Li S, Li YS, Usami S, Ratcliffe A, Wang X, Chien S. Effects of flow patterns on endothelial cell migration into a zone of mechanical denudation. *Biochem Biophys Res Commun*. 2001; 285:751–759. [PubMed: 11453657]
260. Hu YL, Li S, Miao H, Tsou TC, del Pozo MA, Chien S. Roles of microtubule dynamics and small GTPase Rac in endothelial cell migration and lamellipodium formation under flow. *J Vasc Res*. 2002; 39:465–476. [PubMed: 12566972]
261. Huang AL, Jan KM, Chien S. Role of intercellular junctions in the passage of horseradish peroxidase across aortic endothelium. *Lab Invest*. 1992; 67:201–209. [PubMed: 1501446]
262. Hume M. Venous thrombosis: mechanisms and treatment. *Adv Exp Med Biol*. 1978; 102:215–224. [PubMed: 356559]
263. Huo Y, Wischgoll T, Kassab GS. Flow patterns in three-dimensional porcine epicardial coronary arterial tree. *Am J Physiol Heart Circ Physiol*. 2007; 293:H2959–H2970. [PubMed: 17827262]
264. Hutchison KJ. Endothelial cell morphology around graded stenoses of the dog common carotid artery. *Blood Vessels*. 1991; 28:396–406. [PubMed: 1912606]
265. Hwang J, Ing MH, Salazar A, Lassègue B, Griendling K, Navab M, Sevanian A, Hsiai TK. Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circ Res*. 2003; 93:1225–1232. [PubMed: 14593003]
266. Hwang J, Saha A, Boo YC, Sorescu GP, McNally JS, Holland SM, Dikalov S, Giddens DP, Griendling KK, Harrison DG, Jo H. Oscillatory shear stress stimulates endothelial production of O₂⁻ from p47^{phox}-dependent NAD(P)H oxidases, leading to monocyte adhesion. *J Biol Chem*. 2003; 278:47291–47298. [PubMed: 12958309]
267. Iakovou I, Schmidt T, Bonizzoni E, Ge L, Sangiorgi GM, Stankovic G, Airoldi F, Chieffo A, Montorfano M, Carlino M, Michev I, Corvaja N, Briguori C, Gerckens U, Grube E, Colombo A. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA*. 2005; 293:2126–2130. [PubMed: 15870416]
268. Ibegbuna V, Delis KT, Nicolaides AN, Aina O. Effect of elastic compression stockings on venous hemodynamics during walking. *J Vasc Surg*. 2003; 37:420–425. [PubMed: 12563216]
269. Iiyama K, Hajra L, Iiyama M, Li H, DiChiara M, Medoff BD, Cybulsky MI. Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. *Circ Res*. 1999; 85:199–207. [PubMed: 10417402]
270. Illi B, Nanni S, Scopece A, Farsetti A, Biglioli P, Capogrossi MC, Gaetano C. Shear stress-mediated chromatin remodeling provides molecular basis for flow-dependent regulation of gene expression. *Circ Res*. 2003; 93:155–161. [PubMed: 12805238]
271. Ishida M, Komori K, Yonemitsu Y, Taguchi K, Onohara T, Sugimachi K. Immunohistochemical phenotypic alterations of rabbit autologous vein grafts implanted under arterial circulation with

- or without poor distal runoff-implications of vein graft remodeling. *Atherosclerosis*. 2001; 154:345–354. [PubMed: 11166767]
272. Ishida T, Choi SY, Kundu RK, Spin J, Yamashita T, Hirata K, Kojima Y, Yokoyama M, Cooper AD, Quertermous T. Endothelial lipase modulates susceptibility to atherosclerosis in apolipoprotein-E-deficient mice. *J Biol Chem*. 2004; 279:45085–45092. [PubMed: 15304490]
273. Itoh H, Komori K, Funahashi S, Okadome K, Sugimachi K. Intimal hyperplasia of experimental autologous vein graft in hyperlipidemic rabbits with poor distal runoff. *Atherosclerosis*. 1994; 110:259–270. [PubMed: 7848374]
274. Jacob MP, Cazaubon M, Scemama A, Prié D, Blanchet F, Guillin MC, Michel JB. Plasma matrix metalloproteinase-9 as a marker of blood stasis in varicose veins. *Circulation*. 2002; 106:535–538. [PubMed: 12147532]
275. Jalali S, del Pozo MA, Chen K, Miao H, Li Y, Schwartz MA, Shyy JY, Chien S. Integrin-mediated mechanotransduction requires its dynamic interaction with specific extracellular matrix (ECM) ligands. *Proc Natl Acad Sci USA*. 2001; 98:1042–1046. [PubMed: 11158591]
276. Janssens S, Van de Werf F. Cardiology. Acute coronary syndromes: Virchow's triad revisited. *Lancet*. 1996; 348:sII2. [PubMed: 8973480]
277. Jiménez JM, Davies PF. Hemodynamically driven stent strut design. *Ann Biomed Eng*. 2009; 37:1483–1494. [PubMed: 19472055]
278. Jin G, Chieh-Hsi Wu J, Li YS, Hu YL, Shyy JY, Chien S. Effects of active and negative mutants of Ras on rat arterial neointima formation. *J Surg Res*. 2000; 94:124–132. [PubMed: 11104652]
279. Jo H, Song H, Mowbray A. Role of NADPH oxidases in disturbed flow and BMP4-induced inflammation and atherosclerosis. *Antioxid Redox Signal*. 2006; 8:1609–1619. [PubMed: 16987015]
280. Kakkos SK, Zolota VG, Peristeropoulou P, Apostolopoulou A, Geroukalos G, Tsolakis IA. Increased mast cell infiltration in familial varicose veins: pathogenetic implications? *Int Angiol*. 2003; 22:43–49. [PubMed: 12771855]
281. Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am J Physiol Heart Circ Physiol*. 1980; 239:H14–H21.
282. Kamm RD. Cellular fluid mechanics. *Annu Rev Fluid Mech*. 2002; 34:211–232. [PubMed: 12741392]
283. Karino T, Goldsmith HL. Flow behaviour of blood cells and rigid spheres in an annular vortex. *Philos Trans R Soc Lond B Biol Sci*. 1977; 279:413–445. [PubMed: 19795]
284. Karino T, Goldsmith HL. Disturbed flow in models of branching vessels. *Trans Am Soc Artif Intern Organs*. 1980; 26:500–506. [PubMed: 7245540]
285. Karino T, Goldsmith HL. Role of blood cell-wall interactions in thrombogenesis and atherogenesis: a microrheological study. *Biorheology*. 1984; 21:587–601. [PubMed: 6487769]
286. Karino T, Motomiya M. Flow through a venous valve and its implication for thrombus formation. *Thromb Res*. 1984; 36:245–257. [PubMed: 6515603]
287. Kastrati A, Mehilli J, Dirschinger J, Dotzer F, Schühlen H, Neumann FJ, Fleckenstein M, Pfaffert C, Seyfarth M, Schömig A. Intracoronary stenting and angiographic results: strut thickness effect on restenosis outcome (ISAR-STEREO) trial. *Circulation*. 2001; 103:2816–2821. [PubMed: 11401938]
288. Kastrati A, Mehilli J, Dirschinger J, Pache J, Ulm K, Schühlen H, Seyfarth M, Schmitt C, Blasini R, Neumann FJ, Schömig A. Restenosis after coronary placement of various stent types. *Am J Cardiol*. 2001; 87:34–39. [PubMed: 11137830]
289. Kastrati A, Mehilli J, von Beckerath N, Dibra A, Hausleiter J, Pache J, Schühlen H, Schmitt C, Dirschinger J, Schömig A. Study Investigators ISARDESIRE. Sirolimus-eluting stent or paclitaxel-eluting stent vs balloon angioplasty for prevention of recurrences in patients with coronary in-stent restenosis: a randomized controlled trial. *JAMA*. 2005; 293:165–171. [PubMed: 15644543]
290. Khachigian LM, Resnick N, Gimbrone MA Jr, Collins T. Nuclear factor-kappa B interacts functionally with the platelet-derived growth factor B-chain shear-stress response element in vascular endothelial cells exposed to fluid shear stress. *J Clin Invest*. 1995; 96:1169–1175. [PubMed: 7635955]

291. Khatri JJ, Johnson C, Magid R, Lessner SM, Laude KM, Dikalov SI, Harrison DG, Sung HJ, Rong Y, Galis ZS. Vascular oxidant stress enhances progression and angiogenesis of experimental atheroma. *Circulation*. 2004; 109:520–525. [PubMed: 14744973]
292. Kilner PJ, Yang GZ, Mohiaddin RH, Firmin DN, Longmore DB. Helical and retrograde secondary flow patterns in the aortic arch studied by three-directional magnetic resonance velocity mapping. *Circulation*. 1993; 88:2235–2247. [PubMed: 8222118]
293. Kim DW, Gotlieb AI, Langille BL. In vivo modulation of endothelial F-actin microfilaments by experimental alterations in shear stress. *Arteriosclerosis*. 1989; 9:439–445. [PubMed: 2751473]
294. Kim DW, Langille BL, Wong MK, Gotlieb AI. Patterns of endothelial microfilament distribution in the rabbit aorta in situ. *Circ Res*. 1989; 64:21–31. [PubMed: 2909301]
295. Kim YH, Chandran KB, Bower TJ, Corson JD. Flow dynamics across end-to-end vascular bypass graft anastomoses. *Ann Biomed Eng*. 1993; 21:311–320. [PubMed: 8214816]
296. Kinderlerer AR, Ali F, Johns M, Lidington EA, Leung V, Boyle JJ, Hamdulay SS, Evans PC, Haskard DO, Mason JC. KLF2-dependent, shear stress-induced expression of CD59: a novel cytoprotective mechanism against complement-mediated injury in the vasculature. *J Biol Chem*. 2008; 283:14636–14644. [PubMed: 18362151]
297. Kissin M, Kansal N, Pappas PJ, DeFouw DO, Durán WN, Hobson RW 2nd. Vein interposition cuffs decrease the intimal hyperplastic response of polytetrafluoroethylene bypass grafts. *J Vasc Surg*. 2000; 31:69–83. [PubMed: 10642710]
298. Kleinstreuer C, Hyun S, Buchanan JR Jr, Longest PW, Archie JP Jr, Truskey GA. Hemodynamic parameters and early intimal thickening in branching blood vessels. *Crit Rev Biomed Eng*. 2001; 29:1–64. [PubMed: 11321642]
299. Kloner RA, Przyklenk K, Whittaker P. Deleterious effects of oxygen radicals in ischemia/reperfusion. Resolved and unresolved issues. *Circulation*. 1989; 80:1115–1127. [PubMed: 2553296]
300. Kohler TR, Jawien A. Flow affects development of intimal hyperplasia after arterial injury in rats. *Arterioscler Thromb*. 1992; 12:963–971. [PubMed: 1637795]
301. Kohler TR, Kirkman TR, Clowes AW. The effect of rigid external support on vein graft adaptation to the arterial circulation. *J Vasc Surg*. 1989; 9:277–285. [PubMed: 2465421]
302. Kohler TR, Kirkman TR. Dialysis access failure: a sheep model of rapid stenosis. *J Vasc Surg*. 1990; 30:744–751. [PubMed: 10514214]
303. Kohler TR, Toleikis PM, Gravett DM, Avelar RL. Inhibition of neointimal hyperplasia in a sheep model of dialysis access failure with the bioabsorbable Vascular Wrap paclitaxel-eluting mesh. *J Vasc Surg*. 2007; 45:1029–1037. [PubMed: 17466798]
304. Kolachalama VB, Levine EG, Edelman ER. Luminal flow amplifies stent-based drug deposition in arterial bifurcations. *PLoS One*. 2009; 4:e8105. [PubMed: 19956555]
305. Kokura S, Yoshida N, Yoshikawa T. Anoxia/reoxygenation-induced leukocyte-endothelial cell interactions. *Free Radic Biol Med*. 2002; 33:427–432. [PubMed: 12160924]
306. Korshunov VA, Berk BC. Flow-induced vascular remodeling in the mouse: a model for carotid intima-media thickening. *Arterioscler Thromb Vasc Biol*. 2003; 23:2185–2191. [PubMed: 14576075]
307. Korshunov VA, Mohan AM, Georger MA, Berk BC. Axl, a receptor tyrosine kinase, mediates flow-induced vascular remodeling. *Circ Res*. 2006; 98:1446–1452. [PubMed: 16627783]
308. Koslow AR, Stromberg RR, Friedman LI, Lutz RJ, Hilbert SL, Schuster P. A flow system for the study of shear forces upon cultured endothelial cells. *J Biomech Eng*. 1986; 108:338–341. [PubMed: 3795879]
309. Kraiss LW, Geary RL, Mattsson EJR, Vergel S, Au YPT, Clowes AW. Acute reductions in blood flow and shear stress induce platelet-derived growth factor-A expression in baboon prosthetic grafts. *Circ Res*. 1996; 79:45–53. [PubMed: 8925568]
310. Kreienberg PB, Darling RC 3rd, Chang BB, Paty PS, Lloyd WE, Shah DM. Adjunctive techniques to improve patency of distal prosthetic bypass grafts: polytetrafluoroethylene with remote arteriovenous fistulae versus vein cuffs. *J Vasc Surg*. 2000; 31:696–701. [PubMed: 10753277]

311. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. *Arteriosclerosis*. 1985; 5:293–302. [PubMed: 3994585]
312. Kumar A, Lindner V. Remodeling with neointima formation in the mouse carotid artery after cessation of blood flow. *Arterioscler Thromb Vasc Biol*. 1997; 17:2238–2244. [PubMed: 9351395]
313. Kume T, Jiang H, Topczewska JM, Hogan BL. The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and somitogenesis. *Genes Dev*. 2001; 15:2470–2482. [PubMed: 11562355]
314. Kwak BR, Mulhaupt F, Veillard N, Gros DB, Mach F. Altered pattern of vascular connexin expression in atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*. 2002; 22:225–230. [PubMed: 11834520]
315. Kwei S, Stavarakis G, Takahas M, Taylor G, Folkman MJ, Gimbrone MA Jr, García-Cardena G. Early adaptive responses of the vascular wall during venous arterialization in mice. *Am J Pathol*. 2004; 164:81–89. [PubMed: 14695322]
316. Kyrle PA, Eichinger S. Is Virchow's triad complete? *Blood*. 2009; 114:1138–1139. [PubMed: 19661277]
317. Labropoulos N, Delis KT, Nicolaides AN. Venous reflux in symptom-free vascular surgeons. *J Vasc Surg*. 1995; 22:150–154. [PubMed: 7637114]
318. Labropoulos N, Tiongson J, Pryor L, Tassiopoulos AK, Kang SS, Ashraf Mansour M, Baker WH. Definition of venous reflux in the lower extremity veins. *J Vasc Surg*. 2003; 38:793–798. [PubMed: 14560232]
319. Lagattolla NRF, Donald A, Lockhart S, Burnand KG. Retrograde flow in the deep veins of subjects with normal venous function. *Br J Surg*. 1997; 84:36–39. [PubMed: 9043446]
320. Lalka SG, Unthank JL, Nixon JC. Elevated cutaneous leukocyte concentration in a rodent model of acute venous hypertension. *J Surg Res*. 1998; 74:59–63. [PubMed: 9536975]
321. Lan Q, Mercurius KO, Davies PF. Stimulation of transcription factors NF kappa B and AP1 in endothelial cells subjected to shear stress. *Biochem Biophys Res Commun*. 1994; 201:950–956. [PubMed: 8003036]
322. Langille BL, Graham JJ, Kim D, Gotlieb AI. Dynamics of shear-induced redistribution of F-actin in endothelial cells in vivo. *Arterioscler Thromb*. 1991; 11:1814–1820. [PubMed: 1931883]
323. Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science*. 1986; 231:405–407. [PubMed: 3941904]
324. LaPlaca MC, Thibault LE. An in vitro traumatic injury model to examine the response of neurons to a hydrodynamically-induced deformation. *Ann Biomed Eng*. 1997; 25:665–677. [PubMed: 9236979]
325. Laude K, Beauchamp P, Thuillez C, Richard V. Endothelial protective effects of preconditioning. *Cardiovasc Res*. 2002; 55:466–473. [PubMed: 12160943]
326. Lawrence MB, McIntire LV, Eskin SG. Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. *Blood*. 1987; 70:1284–1290. [PubMed: 3663936]
327. Lawson ND, Weinstein BM. Arteries and veins: making a difference with zebrafish. *Nat Rev Genet*. 2002; 3:674–682. [PubMed: 12209142]
328. Leask RL, Jain N, Butany J. Endothelium and valvular diseases of the heart. *Microsc Res Tech*. 2003; 60:129–137. [PubMed: 12539167]
329. Lee JS, Yu Q, Shin JT, Sebzda E, Bertozzi C, Chen M, Mericko P, Stadtfeld M, Zhou D, Cheng L, Graf T, MacRae CA, Lepore JJ, Lo CW, Kahn ML. Klf2 is an essential regulator of vascular hemodynamic forces in vivo. *Dev Cell*. 2006; 11:845–857. [PubMed: 17141159]
330. Lerman A, Burnett JC Jr. Intact and altered endothelium in regulation of vasomotion. *Circulation* 86 Suppl. 1992:III12–III19.
331. Lerman A, Burnett JC, Higano ST, McKinley LJ, Holmes DR. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation*. 1998; 97:2123–2128. [PubMed: 9626172]
332. Levesque MJ, Liepsch D, Moravec S, Nerem RM. Correlation of endothelial cell shape and wall shear stress in a stenosed dog aorta. *Arteriosclerosis*. 1986; 6:220–229. [PubMed: 3954676]

333. Levesque MJ, Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. *J Biomech Eng.* 1985; 107:341–347. [PubMed: 4079361]
334. Levesque MJ, Nerem RM, Sprague EA. Vascular endothelial cell proliferation in culture and the influence of flow. *Biomaterials.* 1990; 11:702–707. [PubMed: 2090307]
335. Li L, Rezvan A, Salerno JC, Husain A, Kwon K, Jo H, Harrison DG, Chen W. GTP cyclohydrolase I phosphorylation and interaction with GTP cyclohydrolase feedback regulatory protein provide novel regulation of endothelial tetrahydrobiopterin and nitric oxide. *Circ Res.* 2010; 106:328–336. [PubMed: 19926872]
336. Li S, Butler P, Wang Y, Hu Y, Han DC, Usami S, Guan JL, Chien S. The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. *Proc Natl Acad Sci USA.* 2002; 99:3546–3551. [PubMed: 11891289]
337. Li YS, Haga JH, Chien S. Molecular basis of the effects of shear stress on vascular endothelial cells. *J Biomech.* 2005; 38:1949–1971. [PubMed: 16084198]
338. Libby P. Inflammation in atherosclerosis. *Nature.* 2002; 420:868–874. [PubMed: 12490960]
339. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation.* 2005; 111:3481–3488. [PubMed: 15983262]
340. Liepsch D. An introduction to biofluid mechanics-basic models and applications. *J Biomech.* 2002; 35:415–435. [PubMed: 11934411]
341. Lieu DK, Pappone PA, Barakat AI. Differential membrane potential and ion current responses to different types of shear stress in vascular endothelial cells. *Am J Physiol Cell Physiol.* 2004; 286:C1367–C1375. [PubMed: 14761889]
342. Lin K, Hsu PP, Chen BP, Yuan S, Usami S, Shyy JY, Li YS, Chien S. Molecular mechanism of endothelial growth arrest by laminar shear stress. *Proc Natl Acad Sci USA.* 2000; 97:9385–9389. [PubMed: 10920209]
343. Lin PH, Bush RL, Nguyen L, Guerrero MA, Chen C, Lumsden AB. Anastomotic strategies to improve hemodialysis access patency: a review. *Vasc Endovascular Surg.* 2005; 39:135–142. [PubMed: 15806274]
344. Lin SJ, Jan KM, Schuessler G, Weinbaum S, Chien S. Enhanced macromolecular permeability of aortic endothelial cells in association with mitosis. *Atherosclerosis.* 1988; 73:223–232. [PubMed: 3190820]
345. Lin SJ, Jan KM, Weinbaum S, Chien S. Transendothelial transport of low density lipoprotein in association with cell mitosis in rat aorta. *Arteriosclerosis.* 1989; 9:230–236. [PubMed: 2923579]
346. Lin Z, Kumar A, SenBanerjee S, Staniszewski K, Parmar K, Vaughan DE, Gimbrone MA Jr, Balasubramanian V, García-Cardena G, Jain MK. Kruppel-like factor 2 (KLF2) regulates endothelial thrombotic function. *Circ Res.* 2005; 96:e48–e57. [PubMed: 15718498]
347. Liu M, Kluger MS, D’Alessio A, García-Cardena G, Pober JS. Regulation of arterial-venous differences in tumor necrosis factor responsiveness of endothelial cells by anatomic context. *Am J Pathol.* 2008; 172:1088–1099. [PubMed: 18292233]
348. Liu SQ. Prevention of focal intimal hyperplasia in rat vein grafts by using a tissue engineering approach. *Atherosclerosis.* 1998; 140:365–377. [PubMed: 9862280]
349. Liu Y, Chen BP, Lu M, Zhu Y, Stemerman MB, Chien S, Shyy JY. Shear stress activation of SREBP1 in endothelial cells is mediated by integrins. *Arterioscler Thromb Vasc Biol.* 2002; 22:76–81. [PubMed: 11788464]
350. Liu Y, Sweet DT, Irani-Tehrani M, Maeda N, Tzima E. Shc coordinates signals from intercellular junctions and integrins to regulate flow-induced inflammation. *J Cell Biol.* 2008; 182:185–196. [PubMed: 18606845]
351. López JA, Kearon C, Lee AY. Deep venous thrombosis. *Hematology Am Soc Hematol Educ Program.* 2004:439–456. [PubMed: 15561697]
352. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med.* 1986; 315:1046–1051. [PubMed: 3093861]
353. Lupu C, Westmuckett AD, Peer G, Ivanciu L, Zhu H, Taylor FB Jr, Lupu F. Tissue factor-dependent coagulation is preferentially up-regulated within arterial branching areas in a baboon model of *Escherichia coli* sepsis. *Am J Pathol.* 2005; 167:1161–1172. [PubMed: 16192650]

354. Lurie F, Kistner RL, Eklof B, Kessler D. Mechanism of venous valve closure and role of the valve in circulation: a new concept. *J Vasc Surg.* 2003; 38:955–961. [PubMed: 14603200]
355. Mack PJ, Zhang Y, Chung S, Vickerman V, Kamm RD, García-Cardena G. Biomechanical regulation of endothelium-dependent events critical for adaptive remodeling. *J Biol Chem.* 2009; 284:8412–8420. [PubMed: 19047056]
356. Magid R, Davies PF. Endothelial protein kinase C isoform identity and differential activity of PKC ζ in an athero-susceptible region of porcine aorta. *Circ Res.* 2005; 97:443–449. [PubMed: 16051884]
357. Magid R, Murphy TJ, Galis ZS. Expression of matrix metalloproteinase-9 in endothelial cells is differentially regulated by shear stress: role of c-Myc. *J Biol Chem.* 2003; 278:32994–32999. [PubMed: 12816956]
358. Maier SE, Meier D, Boesiger P, Moser UT, Vieli A. Human abdominal aorta: comparative measurements of blood flow with MR imaging and multigated Doppler US. *Radiology.* 1989; 171:487–492. [PubMed: 2649924]
359. Makin A, Silverman SH, Lip GY. Peripheral vascular disease and Virchow's triad for thrombogenesis. *QJM.* 2002; 95:199–210. [PubMed: 11937646]
360. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. *JAMA.* 1999; 282:2035–2042. [PubMed: 10591386]
361. Malek AM, Gibbons GH, Dzau VJ, Izumo S. Fluid shear stress differentially modulates expression of genes encoding basic fibroblast growth factor and platelet-derived growth factor B chain in vascular endothelium. *J Clin Invest.* 1993; 92:2013–2021. [PubMed: 8408655]
362. Malinauskas RA, Herrmann RA, Truskey GA. The distribution of intimal white blood cells in the normal rabbit aorta. *Atherosclerosis.* 1995; 115:147–163. [PubMed: 7661874]
363. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui A. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation.* 2000; 101:841–843. [PubMed: 10694520]
364. Margolin DA, Madura JA, de la Motte CA, Fox PL, DiCorleto PE, Graham LM. Differential monocytic cell adherence to specific anatomic regions of the canine aorta. *J Vasc Res.* 1995; 32:266–274. [PubMed: 7654883]
365. Masuda H, Zhuang YJ, Singh TM, Kawamura K, Murakami M, Zarins CK, Glagov S. Adaptive remodeling of internal elastic lamina and endothelial lining during flow-induced arterial enlargement. *Arterioscler Thromb Vasc Biol.* 1999; 19:2298–2307. [PubMed: 10521357]
366. Maulik N, Das DK. Redox signaling in vascular angiogenesis. *Free Radic Biol Med.* 2002; 33:1047–1060. [PubMed: 12374616]
367. Mavromatis K, Fukai T, Tate M, Chesler N, Ku DN, Galis ZS. Early effects of arterial hemodynamic conditions on human saphenous veins perfused ex vivo. *Arterioscler Thromb Vasc Biol.* 2000; 20:1889–1895. [PubMed: 10938008]
368. Mazzolai L, Silacci P, Bouzourene K, Daniel F, Brunner H, Hayoz D. Tissue factor activity is upregulated in human endothelial cells exposed to oscillatory shear stress. *Thromb Haemost.* 2002; 87:1062–1068. [PubMed: 12083487]
369. McCaffrey TA, Fu C, Du B, Eksinar S, Kent KC, Bush H Jr, Kreiger K, Rosengart T, Cybulsky MI, Silverman ES, Collins T. High-level expression of Egr-1 and Egr-1-inducible genes in mouse and human atherosclerosis. *J Clin Invest.* 2000; 105:653–662. [PubMed: 10712437]
370. McCormick SM, Eskin SG, McIntire LV, Teng CL, Lu CM, Russell CG, Chittur KK. DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *Proc Natl Acad Sci USA.* 2001; 98:8955–8960. [PubMed: 11481467]
371. McCue S, Dajnowiec D, Xu F, Zhang M, Jackson MR, Langille BL. Shear stress regulates forward and reverse planar cell polarity of vascular endothelium in vivo and in vitro. *Circ Res.* 2006; 98:939–946. [PubMed: 16527990]
372. McLachlin AD, McLachlin JA, Jory TA, Rawling EG. Venous stasis in the lower extremities. *Ann Surg.* 1960; 152:678–685. [PubMed: 13774059]

373. McLenachan JM, Vita J, Fish DR, Treasure CB, Cox DA, Ganz P, Selwyn AP. Early evidence of endothelial vasodilator dysfunction at coronary branch points. *Circulation*. 1990; 82:1169–1173. [PubMed: 2401058]
374. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol*. 2003; 285:H2290–H2297. [PubMed: 12958034]
375. Melkumyants AM, Balashov SA, Khayutin VM. Endothelium dependent control of arterial diameter by blood viscosity. *Cardiovasc Res*. 1989; 23:741–747. [PubMed: 2611812]
376. Mellor RH, Brice G, Stanton AW, French J, Smith A, Jeffery S, Levick JR, Burnand KG, Mortimer PS. Lymphoedema Research Consortium. Mutations in FOXC2 are strongly associated with primary valve failure in veins of the lower limb. *Circulation*. 2007; 115:1912–1920. [PubMed: 17372167]
377. Methe H, Balcells M, Alegret Mdel C, Santacana M, Molins B, Hamik A, Jain MK, Edelman ER. Vascular bed origin dictates flow pattern regulation of endothelial adhesion molecule expression. *Am J Physiol Heart Circ Physiol*. 2007; 292:H2167–H2175. [PubMed: 17209004]
378. Meyerson SL, Skelly CL, Curi MA, Shakur UM, Vosicky JE, Glagov S, Schwartz LB, Christen T, Gabbiani G. The effects of extremely low shear stress on cellular proliferation and neointimal thickening in the failing bypass graft. *J Vasc Surg*. 2001; 34:90–97. [PubMed: 11436080]
379. Miao H, Hu YL, Shiu YT, Yuan S, Zhao Y, Kaunas R, Wang Y, Jin G, Usami S, Chien S. Effects of flow patterns on the localization and expression of VE-cadherin at vascular endothelial cell junctions: in vivo and in vitro investigations. *J Vasc Res*. 2005; 42:77–89. [PubMed: 15637443]
380. Michiels C, Bouaziz N, Remacle J. Role of the endothelium and blood stasis in the development of varicose veins. *Int Angiol*. 2002; 21:18–25. [PubMed: 12515976]
381. Miller JH, Foreman RK, Ferguson L, Faris I. Interposition vein cuff for anastomosis of prosthesis to small artery. *Aust N Z J Surg*. 1984; 54:283–285. [PubMed: 6380479]
382. Mills NL, Ochsner JL. Valvulotomy of valves in the saphenous vein graft before coronary artery bypass. *J Thorac Cardiovasc Surg*. 1976; 71:878–879. [PubMed: 1083926]
383. Min W, Pober JS. TNF initiates E-selectin transcription in human endothelial cells through parallel TRAF-NF-kappa B and TRAF-RAC/CDC42-JNK-c-Jun/ATF2 pathways. *J Immunol*. 1997; 159:3508–3518. [PubMed: 9317150]
384. Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG. Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. *J Clin Invest*. 1990; 86:2109–2116. [PubMed: 2254462]
385. Miriyala S, Gongora Nieto MC, Mingone C, Smith D, Dikalov S, Harrison DG, Jo H. Bone morphogenic protein-4 induces hypertension in mice: role of noggin, vascular NADPH oxidases, and impaired vasorelaxation. *Circulation*. 2006; 113:2818–2825. [PubMed: 16769910]
386. Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA, Kajiya F. Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release. *Am J Physiol Heart Circ Physiol*. 2003; 285:H722–H726. [PubMed: 12730059]
387. Mohan S, Mohan N, Sprague EA. Differential activation of NFkappa B in human aortic endothelial cells conditioned to specific flow environments. *Am J Physiol Cell Physiol*. 1997; 273:C572–C578.
388. Mohan S, Mohan N, Valente AJ, Sprague EA. Regulation of low shear flow-induced HAEC VCAM-1 expression and monocyte adhesion. *Am J Physiol Cell Physiol*. 1999; 276:C1100–C1107.
389. Mohler ER 3rd, Gannon F, Reynolds C, Zimmerman R, Keane MG, Kaplan FS. Bone formation and inflammation in cardiac valves. *Circulation*. 2001; 103:1522–1528. [PubMed: 11257079]
390. Montgomery H, Zintel HA. Clinical study and treatment of varicose veins. *Circulation*. 1954; 10:442–450. [PubMed: 13190616]
391. Montenegro MR, Eggen DA. Topography of atherosclerosis in the coronary arteries. *Lab Invest*. 1968; 18:586–593. [PubMed: 5681200]

392. Moore JE Jr, Bürki E, Suci A, Zhao S, Burnier M, Brunner HR, Meister JJ. A device for subjecting vascular endothelial cells to both fluid shear stress and circumferential cyclic stretch. *Ann Biomed Eng.* 1994; 22:416–422. [PubMed: 7998687]
393. Moore JE Jr, Xu C, Glagov S, Zarins CK, Ku DN. Fluid wall shear stress measurements in a model of the human abdominal aorta: oscillatory behavior and relationship to atherosclerosis. *Atherosclerosis.* 1994; 110:225–240. [PubMed: 7848371]
394. Moses JW, Leon MB, Popma JJ, Fitzgerald PJ, Holmes DR, O’Shaughnessy C, Caputo RP, Kereiakes DJ, Williams DO, Teirstein PS, Jaeger JL, Kuntz RE. Investigators SIRIUS. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. *N Engl J Med.* 2003; 349:1315–1323. [PubMed: 14523139]
395. Mowbray AL, Kang DH, Rhee SG, Kang SW, Jo H. Laminar shear stress up-regulates peroxiredoxins (PRX) in endothelial cells: PRX 1 as a mechanosensitive antioxidant. *J Biol Chem.* 2008; 283:1622–1627. [PubMed: 18024958]
396. Mózes, G.; Carmichael, SW.; Gloviczki, P. Development and anatomy of the venous system. In: Gloviczki, P.; Yao, JS., editors. *Handbook of Venous Disorders.* 2nd ed. New York, NY: Arnold; 2001. p. 11-24.
397. Mueller CF, Widder JD, McNally JS, McCann L, Jones DP, Harrison DG. The role of the multidrug resistance protein-1 in modulation of endothelial cell oxidative stress. *Circ Res.* 2005; 97:637–644. [PubMed: 16123331]
398. Muller AM, Cronen C, Kupferwasser LI. Expression of endothelial cell adhesion molecules on heart valves: up-regulation in defeneration as well as acute endocarditis. *J Pathol.* 2000; 191:54–60. [PubMed: 10767719]
399. Mullick AE, Soldau K, Kiosses WB, Bell TA 3rd, Tobias PS, Curtiss LK. Increased endothelial expression of Toll-like receptor 2 at sites of disturbed blood flow exacerbates early atherogenic events. *J Exp Med.* 2008; 205:373–383. [PubMed: 18250194]
400. Mullick AE, Tobias PS, Curtiss LK. Modulation of atherosclerosis in mice by Toll-like receptor 2. *J Clin Invest.* 2005; 115:3149–3156. [PubMed: 16211093]
401. Myers DD, Hawley AE, Farris DM, Wroblewski SK, Thanaporn P, Schaub RG, Wagner DD, Kumar A, Wakefield TW. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. *J Vasc Surg.* 2003; 38:1075–1089. [PubMed: 14603220]
402. Myers DD Jr, Henke PK, Bedard PW, Wroblewski SK, Kaila N, Shaw G, Meier TR, Hawley AE, Schaub RG, Wakefield TW. Treatment with an oral small molecule inhibitor of P selectin (PSI-697) decreases vein wall injury in a rat stenosis model of venous thrombosis. *J Vasc Surg.* 2006; 44:625–632. [PubMed: 16950445]
403. Myers DD, Wakefield TW. Inflammation-dependent thrombosis. *Front Biosci.* 2005; 10:2750–2757. [PubMed: 15970530]
404. Myers D Jr, Farris D, Hawley A, Wroblewski S, Chapman A, Stoolman L, Knibbs R, Strieter R, Wakefield T. Selectins influence thrombosis in a mouse model of experimental deep venous thrombosis. *J Surg Res.* 2002; 108:212–221. [PubMed: 12505044]
405. Nagel T, Resnick N, Dewey CF, Gimbrone MA Jr. Vascular endothelial cells respond to spatial gradients in fluid shear stress by enhanced activation of transcription factors. *Arterioscler Thromb Vasc Biol.* 1999; 19:1825–1834. [PubMed: 10446060]
406. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb.* 1994; 14:133–140. [PubMed: 8274468]
407. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol.* 1998; 18:842–851. [PubMed: 9598845]
408. Nakazawa G, Finn AV, Virmani R. Drug-eluting stent pathology-should we still be cautious? *Nat Clin Pract Cardiovasc Med.* 2008; 5:1. [PubMed: 18094669]
409. Nakazawa G, Yazdani SK, Finn AV, Vorpahl M, Kolodgie FD, Virmani R. Pathological findings at bifurcation lesions: the impact of flow distribution on atherosclerosis and arterial healing after stent implantation. *J Am Coll Cardiol.* 2010; 55:1679–1687. [PubMed: 20394871]

410. Nam D, Ni CW, Rezvan A, Suo J, Budzyn K, Llanos A, Harrison DG, Giddens D, Jo H. Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. *Am J Physiol Heart Circ Physiol.* 2009; 297:H1535–H1543. [PubMed: 19684185]
411. Negishi M, Lu D, Zhang YQ, Sawada Y, Sasaki T, Kayo T, Ando J, Izumi T, Kurabayashi M, Kojima I, Masuda H, Takeuchi T. Upregulatory expression of furin and transforming growth factor-beta by fluid shear stress in vascular endothelial cells. *Arterioscler Thromb Vasc Biol.* 2001; 21:785–790.
412. Neglen P, Egger JF, Olivier J, Raju S. Hemodynamic and clinical impact of ultrasound-derived venous reflux parameters. *J Vasc Surg.* 2004; 40:303–310. [PubMed: 15297826]
413. Neglén P, Hollis KC, Olivier J, Raju S. Stenting of the venous outflow in chronic venous disease: long-term stent-related outcome, clinical, and hemodynamic result. *J Vasc Surg.* 2007; 46:979–990. [PubMed: 17980284]
414. Neglén P, Hollis KC, Raju S. Combined saphenous ablation and iliac stent placement for complex severe chronic venous disease. *J Vasc Surg.* 2006; 44:828–833. [PubMed: 17012006]
415. Neglén P, Thrasher TL, Raju S. Venous outflow obstruction: an underestimated contributor to chronic venous disease. *J Vasc Surg.* 2003; 38:879–885. [PubMed: 14603188]
416. Nerem RM. Vascular fluid mechanics, the arterial wall, and atherosclerosis. *J Biomech Eng.* 1992; 114:274–282. [PubMed: 1522720]
417. Nerem RM, Alexander RW, Chappell DC, Medford RM, Varner SE, Taylor WR. The study of the influence of flow on vascular endothelial biology. *Am J Med Sci.* 1998; 316:169–175. [PubMed: 9749558]
418. Nerem RM, Seliktar D. Vascular tissue engineering. *Annu Rev Biomed Eng.* 2001; 3:225–243. [PubMed: 11447063]
419. Neville RF, Attinger C, Sidawy AN. Prosthetic bypass with a distal vein patch for limb salvage. *Am J Surg.* 1997; 174:173–176. [PubMed: 9293838]
420. Newman MF, Kirchner JL, Phillips-Bute B, Gaver V, Grocott H, Jones RH, Mark DB, Reves JG, Blumenthal JA. Neurological Outcome Research Group; Cardiothoracic Anesthesiology Research Endeavors Investigators. Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. *N Engl J Med.* 2001; 344:395–402. [PubMed: 11172175]
421. Ng MY, Andrew T, Spector TD, Jeffery S. Lymphoedema Consortium. Linkage to the FOXC2 region of chromosome 16 for varicose veins in otherwise healthy, unselected sibling pairs. *J Med Genet.* 2005; 42:235–239. [PubMed: 15744037]
422. Ni CW, Hsieh HJ, Chao YJ, Wang DL. Interleukin-6-induced JAK2/STAT3 signaling pathway in endothelial cells is suppressed by hemodynamic flow. *Am J Physiol Cell Physiol.* 2004; 287:C771–C780. [PubMed: 15151905]
423. Nicolaides AN. Investigation of chronic venous insufficiency: a consensus statement (France, March 5–9, 1997). *Circulation.* 2000; 102:E126–E163. [PubMed: 11076834]
424. Nicolaides AN. Chronic venous disease and the leukocyte-endothelium interaction: from symptoms to ulceration. *Angiology.* 2005; 56:S11–S19. [PubMed: 16193221]
425. Nicosia MA, Cochran RP, Einstein DR, Rutland CJ, Kunzelman KS. A coupled fluid-structure finite element model of the aortic valve and root. *J Heart Valve Dis.* 2003; 12:781–789. [PubMed: 14658821]
426. Niebauer J, Cooke JP. Cardiovascular effects of exercise: role of endothelial shear stress. *J Am Coll Cardiol.* 1996; 28:1652–1660. [PubMed: 8962548]
427. Niebauer J, Maxwell AJ, Lin PS, Wang D, Tsao PS, Cooke JP. NOS inhibition accelerates atherogenesis: reversal by exercise. *Am J Physiol Heart Circ Physiol.* 2003; 285:H535–H540. [PubMed: 12598230]
428. Nieuwdorp M, Meuwese MC, Vink H, Hoekstra JB, Kastelein JJ, Stroes ES. The endothelial glycocalyx: a potential barrier between health and vascular disease. *Curr Opin Lipidol.* 2005; 16:507–511. [PubMed: 16148534]
429. Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, Langer R. Functional arteries grown in vitro. *Science.* 1999; 284:489–493. [PubMed: 10205057]

430. Nilsen TW. Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends Genet.* 2007; 23:243–249. [PubMed: 17368621]
431. Noris M, Morigi M, Donadelli R, Aiello S, Foppolo M, Todeschini M, Orisio S, Remuzzi G, Remuzzi A. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circ Res.* 1995; 76:536–543. [PubMed: 7534657]
432. O'Brien KD, Reichenbach DD, Marcovina SM, Kuusisto J, Alpers CE, Otto CM. Apolipoproteins B, (a), and E accumulate in the morphologically early lesion of “degenerative” valvular aortic stenosis. *Arterioscler Thromb Vasc Biol.* 1996; 16:523–532. [PubMed: 8624774]
433. Oemar BS, Werner A, Garnier JM, Do DD, Godoy N, Nauck M, Marz W, Rupp J, Pech M, Luscher TF. Human connective tissue growth factor is expressed in advanced atherosclerotic lesions. *Circulation.* 1997; 95:831–839. [PubMed: 9054739]
434. Ojha M. Wall shear stress temporal gradient and anastomotic intimal hyperplasia. *Circ Res.* 1994; 74:1227–1231. [PubMed: 8187288]
435. Oliviero U, Bonadies G, Apuzzi V, Foggia M, Bosso G, Nappa S, Valvano A, Leonardi E, Borgia G, Castello G, Napoli R, Saccà L. Human immunodeficiency virus per se exerts atherogenic effects. *Atherosclerosis.* 2009; 204:586–589. [PubMed: 19084229]
436. Ono T, Bergan JJ, Schmid-Schonbein GW, Takase S. Monocyte infiltration into venous valves. *J Vasc Surg.* 1998; 27:158–166. [PubMed: 9474094]
437. Orr AW, Hahn C, Blackman BR, Schwartz MA. p21-activated kinase signaling regulates oxidant-dependent nuclear factor κ B activation by flow. *Circ Res.* 2008; 103:671–679. [PubMed: 18669917]
438. Orr AW, Helmke BP, Blackman BR, Schwartz MA. Mechanisms of mechanotransduction. *Dev Cell.* 2006; 10:11–20. [PubMed: 16399074]
439. Orr AW, Sanders JM, Bevard M, Coleman E, Sarembock IJ, Schwartz MA. The subendothelial extracellular matrix modulates NF- κ B activation by flow: a potential role in atherosclerosis. *J Cell Biol.* 2005; 169:191–202. [PubMed: 15809308]
440. Orr AW, Stockton R, Simmers MB, Sanders JM, Sarembock IJ, Blackman BR, Schwartz MA. Matrix-specific p21-activated kinase activation regulates vascular permeability in atherogenesis. *J Cell Biol.* 2007; 176:719–727. [PubMed: 17312022]
441. Otto CM, Kuusisto J, Reichenbach DD, Gown AM, O'Brien KD. Characterization of the early lesion of “degenerative” valvular aortic stenosis. Histological and immunohistochemical studies. *Circulation.* 1994; 90:844–853. [PubMed: 7519131]
442. Palermo LP, Reid JD, Chan AM, Lokanathan R, Macdonald S, Sladen JG. Results of prosthetic-vein composite graft with remote popliteal arteriovenous fistula in infragenicular bypass. *J Vasc Surg.* 2002; 36:330–335. [PubMed: 12170214]
443. Pappas PJ, DeFouw DO, Venezio LM, Gorti R, Padberg FT Jr, Silva MB Jr, Goldberg MC, Durán WN, Hobson RW 2nd. Morphometric assessment of the dermal microcirculation in patients with chronic venous insufficiency. *J Vasc Surg.* 1997; 26:784–795. [PubMed: 9372816]
444. Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, Kratz JR, Lin Z, Jain MK, Gimbrone MA Jr, García-Cardeña G. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest.* 2006; 116:49–58. [PubMed: 16341264]
445. Partridge J, Carlsen H, Enesa K, Chaudhury H, Zakkar M, Luong L, Kinderlerer A, Johns M, Blomhoff R, Mason JC, Haskard DO, Evans PC. Laminar shear stress acts as a switch to regulate divergent functions of NF- κ B in endothelial cells. *FASEB J.* 2007; 21:3553–3561. [PubMed: 17557931]
446. Pascarella L, Lulic D, Penn AH, Alsaigh T, Lee J, Shin H, Kapur V, Bergan JJ, Schmid-Schönbein GW. Mechanisms in experimental venous valve failure and their modification by Daflon-500 mg. *Eur J Vasc Endovasc Surg.* 2008; 35:102–110. [PubMed: 17890112]
447. Pascarella L, Penn A, Schmid-Schönbein GW. Venous hypertension and the inflammatory cascade: major manifestations and trigger mechanisms. *Angiology.* 2005; 56:S3–S10. [PubMed: 16193224]
448. Pascarella L, Schmid-Schönbein GW. Causes of telangiectasias, reticular veins, and varicose veins. *Semin Vasc Surg.* 2005; 18:2–4. [PubMed: 15791545]

449. Pascarella L, Schmid-Schönbein GW, Bergan J. An animal model of venous hypertension: the role of inflammation in venous valve failure. *J Vasc Surg.* 2005; 41:303–311. [PubMed: 15768014]
450. Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, Pritchard WF, Powell S, Chang GY, Stoeckert CJ Jr, Davies PF. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. *Proc Natl Acad Sci USA.* 2004; 101:2482–2487. [PubMed: 14983035]
451. Paszkowiak J, Dardik A. Arterial wall shear stress: observations from the bench to the bedside. *Vasc Endovasc Surg.* 2003; 37:47–57.
452. Paterson JC, McLachlin J. Precipitating factors in venous thrombosis. *Surg Gynecol Obstet.* 1954; 98:96–102. [PubMed: 13122392]
453. Pearce MJ, McIntyre TM, Prescott SM, Zimmerman GA, Whatley RE. Shear stress activates cytosolic phospholipase A₂ (cPLA₂) and MAP kinase in human endothelial cells. *Biochem Biophys Res Commun.* 1996; 218:500–504. [PubMed: 8561785]
454. Pedersen EM, Agerbaek M, Kristensen IB, Yoganathan AP. Wall shear stress and early atherosclerotic lesions in the abdominal aorta in young adults. *Eur J Vasc Endovasc Surg.* 1997; 13:443–451. [PubMed: 9166266]
455. Pedersen EM, Oyre S, Agerbaek M, Kristensen IB, Ringgaard S, Boesiger P, Paaske WP. Distribution of early atherosclerotic lesions in the human abdominal aorta correlates with wall shear stresses measured in vivo. *Eur J Vasc Endovasc Surg.* 1999; 18:328–333. [PubMed: 10550268]
456. Peng X, Recchia FA, Byrne BJ, Wittstein IS, Ziegelstein RC, Kass DA. In vitro system to study realistic pulsatile flow and stretch signaling in cultured vascular cells. *Am J Physiol Cell Physiol.* 2000; 279:C797–C805. [PubMed: 10942730]
457. Petrova TV, Karpanen T, Norrmen C, Mellor R, Tamakoshi T, Finegold D, Ferrell R, Kerjaschki D, Mortimer P, Yla-Herttuala S, Miura N, Alitalo K. Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. *Nat Med.* 2004; 10:974–981. [PubMed: 15322537]
458. Phelps JE, DePaola N. Spatial variations in endothelial barrier function in disturbed flows in vitro. *Am J Physiol Heart Circ Physiol.* 2000; 278:H469–H476. [PubMed: 10666077]
459. Pi X, Yan C, Berk BC. Big mitogen-activated protein kinase (BMK1)/ERK5 protects endothelial cells from apoptosis. *Circ Res.* 2004; 94:362–369. [PubMed: 14670836]
460. Pinsky DJ. The vascular biology of heart and lung preservation for transplantation. *Thromb Haemost.* 1995; 74:58–65. [PubMed: 8578526]
461. Platt MO, Ankeny RF, Shi GP, Weiss D, Vega JD, Taylor WR, Jo H. Expression of cathepsin K is regulated by shear stress in cultured endothelial cells and is increased in endothelium in human atherosclerosis. *Am J Physiol Heart Circ Physiol.* 2007; 292:H1479–H1486. [PubMed: 17098827]
462. Poelmann RE, Van der Heiden K, Gittenberger-de Groot A, Hierck BP. Deciphering the endothelial shear stress sensor. *Circulation.* 2008; 117:1124–1126. [PubMed: 18316496]
463. Poggianti E, Venneri L, Chubuchny V, Jambrik Z, Baroncini LA, Picano E. Aortic valve sclerosis is associated with systemic endothelial dysfunction. *J Am Coll Cardiol.* 2003; 41:136–141. [PubMed: 12570956]
464. Pohl U, De Wit C, Gloe T. Large arterioles in the control of blood flow: role of endothelium-dependent dilation. *Acta Physiol Scand.* 2000; 168:505–510. [PubMed: 10759587]
465. Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension.* 1986; 8:37–44. [PubMed: 3080370]
466. Polacek D, Bech F, McKinsey JF, Davies PF. Connexin43 gene expression in the rabbit arterial wall: effects of hypercholesterolemia, balloon injury and their combination. *J Vasc Res.* 1997; 34:19–30. [PubMed: 9075822]
467. Polacek D, Lal R, Volin MV, Davies PF. Gap junctional communication between vascular cells. Induction of connexin43 messenger RNA in macrophage foam cells of atherosclerotic lesions. *Am J Pathol.* 1993; 142:593–606.

468. Pollock JS, Nakane M, Buttery LD, Martinez A, Springall D, Polak JM, Förstermann U, Murad F. Characterization and localization of endothelial nitric oxide synthase using specific monoclonal antibodies. *Am J Physiol.* 1993; 265:C1379–C1387. [PubMed: 7694497]
469. Porat RM, Grunewald M, Globerman A, Itin A, Barshtein G, Alhonen L, Alitalo K, Keshet E. Specific induction of tie1 promoter by disturbed flow in atherosclerosis-prone vascular niches and flow-obstructing pathologies. *Circ Res.* 2004; 94:394–401. [PubMed: 14670840]
470. Potter DR, Damiano ER. The hydrodynamically relevant endothelial cell glycocalyx observed in vivo is absent in vitro. *Circ Res.* 2008; 102:770–776. [PubMed: 18258858]
471. Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? *J Appl Physiol.* 2004; 97:1119–1128. [PubMed: 15333630]
472. Qin F, Dardik H, Pangilinan A, Robinson J, Chuy J, Wengerter K. Remodeling and suppression of intimal hyperplasia of vascular grafts with a distal arteriovenous fistula in a rat model. *J Vasc Surg.* 2001; 34:701–706. [PubMed: 11668327]
473. Qin X, Wang X, Wang Y, Tang Z, Cui Q, Xi J, Li YS, Chien S, Wang N. MicroRNA-19a mediates the suppressive effect of laminar flow on cyclin D1 expression in human umbilical vein endothelial cells. *Proc Natl Acad Sci USA.* 2010; 107:3240–3244. [PubMed: 20133739]
474. Qiu Y, Tarbell JM. Interaction between wall shear stress and circumferential strain affects endothelial cell biochemical production. *J Vasc Res.* 2000; 37:147157.
475. Qui Y, Quijano RC, Wang SK, Hwang NH. Fluid dynamics of venous valve closure. *Ann Biomed Eng.* 1995; 23:750–759. [PubMed: 8572425]
476. Raghavan ML, Vorp DA, Federle MP, Makaroun MS, Webster MW. Wall stress distribution on three-dimensionally reconstructed models of human abdominal aortic aneurysm. *J Vasc Surg.* 2000; 31:760–769. [PubMed: 10753284]
477. Raju S, Hollis K, Neglen P. Obstructive lesions of the inferior vena cava: clinical features and endovenous treatment. *J Vasc Surg.* 2006; 44:820–827. [PubMed: 16926084]
478. Raju S, Neglén P. Clinical practice: chronic venous insufficiency and varicose veins. *N Engl J Med.* 2009; 360:2319–2327. [PubMed: 19474429]
479. Rand JH, Badimon L, Gordon RE, Uson RR, Fuster V. Distribution of vWF in porcine intima varies with blood vessel type and location. *Arteriosclerosis.* 1987; 7:287–291. [PubMed: 3297010]
480. Read MA, Whitley MZ, Gupta S, Pierce JW, Best J, Davis RJ, Collins T. Tumor necrosis factor alpha-induced E-selectin expression is activated by the nuclear factor-kappaB and c-JUN N-terminal kinase/p38 mitogen-activated protein kinase pathways. *J Biol Chem.* 1997; 272:2753–2761. [PubMed: 9006914]
481. Rectenwald JE, Minter RM, Moldawer LL, Abouhamze Z, La Face D, Hutchins E, Huber TS, Seeger JM, Ozaki CK. Interleukin-10 fails to modulate low shear stress-induced neointimal hyperplasia. *J Surg Res.* 2002; 102:110–118. [PubMed: 11796006]
482. Redmond EM, Cahill PA, Sitzmann JV. Perfused transcapillary smooth muscle and endothelial cell co-culture—a novel in vitro model. *In Vitro. Cell Dev Biol Anim.* 1995; 31:601–609.
483. Reidy MA, Langille BL. The effect of local blood flow patterns on endothelial cell morphology. *Exp Mol Pathol.* 1980; 32:276–289. [PubMed: 7379981]
484. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflügers Arch.* 2007; 454:345–359.
485. Resnick N, Gimbrone MA Jr. Hemodynamic forces are complex regulators of endothelial gene expression. *FASEB J.* 1995; 9:874–882. [PubMed: 7615157]
486. Resnick N, Yahav H, Shay-Salit A, Shushy M, Schubert S, Zilberman LC, Wofovitz E. Fluid shear stress and the vascular endothelium: for better and for worse. *Prog Biophys Mol Biol.* 2003; 81:177–199. [PubMed: 12732261]
487. Rhee WJ, Ni CW, Zheng Z, Chang K, Jo H, Bao G. HuR regulates the expression of stress-sensitive genes and mediates inflammatory response in human umbilical vein endothelial cells. *Proc Natl Acad Sci USA.* 2010; 107:6858–6863. [PubMed: 20351266]
488. Ricci R, Sumara G, Sumara I, Rozenberg I, Kurrer M, Akhmedov A, Hersberger M, Eriksson U, Eberli FR, Becher B, Borén J, Chen M, Cybulsky MI, Moore KJ, Freeman MW, Wagner EF,

- Matter CM, Lüscher TF. Requirement of JNK2 for scavenger receptor A-mediated foam cell formation in atherogenesis. *Science*. 2004; 306:1558–1561. [PubMed: 15567863]
489. Richter Y, Groothuis A, Seifert P, Edelman ER. Dynamic flow alterations dictate leukocyte adhesion and response to endovascular interventions. *J Clin Invest*. 2003; 113:1607–1614. [PubMed: 15173887]
490. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. *Biochem Pharmacol*. 1988; 37:837–841. [PubMed: 2830882]
491. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993; 362:801–809. [PubMed: 8479518]
492. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med*. 1999; 340:115–126. [PubMed: 9887164]
493. Ross R, Glomset J. The pathogenesis of atherosclerosis. *N Engl J Med*. 1976; 295:369–377. [PubMed: 819830]
494. Sabbah HN, Khaja F, Brymer JF, Hawkins ET, Stein PD. Blood velocity in the right coronary: relation to the distribution of atherosclerotic lesions. *Am J Cardiol*. 1984; 53:1008–1012. [PubMed: 6702675]
495. Sacks MS, Yoganathan AP. Heart valve function: a biomechanical perspective. *Philos Trans R Soc Lond B Biol Sci*. 2007; 362:1369–1391. [PubMed: 17588873]
496. Safar P. Cerebral resuscitation after cardiac arrest: a review. *Circulation*. 1986; 74:IV138–IV153. [PubMed: 3536160]
497. Saharay M, Shields DA, Georgiannos SN, Porter JB, Scurr JH, Coleridge Smith PD. Endothelial activation in patients with chronic venous disease. *Eur J Vasc Endovasc Surg*. 1998; 15:342–349. [PubMed: 9610348]
498. Salsac AV, Sparks SR, Chomaz JM, Lasheras JC. Evolution of the wall shear stresses during the progressive enlargement of symmetric abdominal aortic aneurysms. *J Fluid Mech*. 2006; 560:19–51.
499. Sanmartín M, Goicolea J, García C, García J, Crespo A, Rodríguez J, Goicolea JM. Influence of shear stress on in-stent restenosis: in vivo study using 3D reconstruction and computational fluid dynamics. *Rev Esp Cardiol*. 2006; 59:20–27. [PubMed: 16434000]
500. Sarin S, Sommerville K, Farrah J, Scurr JH, Coleridge Smith PD. Duplex ultrasonography for assessment of venous valvular function of the lower limb. *Br J Surg*. 1994; 81:1591–1595. [PubMed: 7827879]
501. Satoh K, Matoba T, Suzuki J, O'Dell MR, Nigro P, Cui Z, Mohan A, Pan S, Li L, Jin ZG, Yan C, Abe J, Berk BC. Cyclophilin A mediates vascular remodeling by promoting inflammation and vascular smooth muscle cell proliferation. *Circulation*. 2008; 117:3088–3098. [PubMed: 18541741]
502. Sawchuk AP, Unthank JL, Davis TE, Dalsing MC. A prospective, in vivo study of the relationship between blood flow hemodynamics and atherosclerosis in a hyperlipidemic swine model. *J Vasc Surg*. 1994; 19:58–64. [PubMed: 8301738]
503. Schmauss D, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation*. 2008; 117:2131–2141. [PubMed: 18427143]
504. Schnittler HJ, Franke RP, Akbay U, Mrowietz C, Drenckhahn D. Improved in vitro rheological system for studying the effect of fluid shear stress on cultured cells. *Am J Physiol Cell Physiol*. 1993; 265:C289–C298.
505. Schuler G, Hambrecht R, Schlierf G, Niebauer J, Hauer K, Neumann J, Hoberg E, Drinkmann A, Bacher F, Grunze M. Regular physical exercise and low-fat diet. Effects on progression of coronary artery disease. *Circulation*. 1992; 86:1–11. [PubMed: 1617762]
506. Schwenke DC, Carew TE. Quantification in vivo of increased LDL content and rate of LDL degradation in normal rabbit aorta occurring at sites susceptible to early atherosclerotic lesions. *Circ Res*. 1988; 62:699–710. [PubMed: 3349573]
507. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. *Arteriosclerosis*. 1989; 9:895–907. [PubMed: 2590067]

508. Seifert PS, Hugo F, Hansson GK, Bhakdi S. Prelesional complement activation in experimental atherosclerosis. Terminal C5b-9 complement deposition coincides with cholesterol accumulation in the aortic intima of hypercholesterolemic rabbits. *Lab Invest.* 1989; 60:747–754. [PubMed: 2659887]
509. SenBanerjee S, Lin Z, Atkins GB, Greif DM, Rao RM, Kumar A, Feinberg MW, Chen Z, Simon DI, Luscsinkas FW, Michel TM, Gimbrone MA Jr, García-Cardena G, Jain MK. KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. *J Exp Med.* 2004; 199:1305–1301. [PubMed: 15136591]
510. Sengoelge G, Luo W, Fine D, Perschl AM, Fierlbeck W, Haririan A, Sorensson J, Rehman TU, Hauser P, Trevick JS, Kulak SC, Wegner B, Ballermann BJ. A SAGE-based comparison between glomerular and aortic endothelial cells. *Am J Physiol Renal Physiol.* 2005; 288:F1290–F1300. [PubMed: 15657302]
511. Serruys PW, de Jaegere P, Kiemeneij F, Macaya C, Rutsch W, Heyndrickx G, Emanuelsson H, Marco J, Legrand V, Materne P. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. Benestent Study Group. *N Engl J Med.* 1994; 331:489–495. [PubMed: 8041413]
512. Sevitt S. The structure and growth of valve-pocket thrombi in femoral veins. *J Clin Pathol.* 1974; 27:517–528. [PubMed: 4138834]
513. Sharp DL, Donovan WV, Teague PC, Mosteller RD. Arterial occlusive disease: a function of vessel bifurcation angle. *Surgery.* 1982; 91:680–685. [PubMed: 7079970]
514. Shimogonya Y, Ishikawa T, Imai Y, Matsuki N, Yamaguchi T. Can temporal fluctuation in spatial wall shear stress gradient initiate a cerebral aneurysm? A proposed novel hemodynamic index, the gradient oscillatory number (GON). *J Biomech.* 2009; 42:550–554. [PubMed: 19195658]
515. Shishehbor MH, Aviles RJ, Brennan ML, Fu X, Goormastic M, Pearce GL, Gokce N, Keaney JF Jr, Penn MS, Sprecher DL, Vita JA. Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy. *JAMA.* 2003; 289:1675–1680. [PubMed: 12672736]
516. Sho E, Sho M, Singh TM, Nanjo H, Komatsu M, Xu C, Masuda H, Zarins CK. Arterial enlargement in response to high flow requires early expression of matrix metalloproteinases to degrade extracellular matrix. *Exp Mol Pathol.* 2002; 73:142–153. [PubMed: 12231217]
517. Sho E, Sho M, Singh TM, Xu C, Zarins CK, Masuda H. Blood flow decrease induces apoptosis of endothelial cells in previously dilated arteries resulting from chronic high blood flow. *Arterioscler Thromb Vasc Biol.* 2001; 21:1139–1145. [PubMed: 11451742]
518. Sho M, Sho E, Singh TM, Komatsu M, Sugita A, Xu C, Nanjo H, Zarins CK, Masuda H. Subnormal shear stress-induced intimal thickening requires medial smooth muscle cell proliferation and migration. *Exp Mol Pathol.* 2002; 72:150–160. [PubMed: 11890724]
519. Shoab SS, Scurr JH, Coleridge-Smith PD. Increased plasma vascular endothelial growth factor among patients with chronic venous disease. *J Vasc Surg.* 1998; 28:535–540. [PubMed: 9737465]
520. Shyy JY. Mechanotransduction in endothelial responses to shear stress: review of work in Dr. Chien's laboratory. *Biorheology.* 2001; 38:109–117. [PubMed: 11381169]
521. Shyy JY. Extracellular matrix differentiating good flow versus bad flow. *Circ Res.* 2009; 104:931–932. [PubMed: 19390061]
522. Shyy JY, Chien S. Role of integrins in endothelial mechanosensing of shear stress. *Circ Res.* 2002; 91:769–775. [PubMed: 12411390]
523. Shyy JY, Hsieh HJ, Usami S, Chien S. Fluid shear stress induces a biphasic response of human monocyte chemoattractant protein 1 gene expression in vascular endothelium. *Proc Natl Acad Sci USA.* 1994; 91:4678–4682. [PubMed: 8197118]
524. Shyy JY, Li YS, Lin MC, Chen W, Yuan S, Usami S, Chien S. Multiple *cis*-elements mediate shear stress-induced gene expression. *J Biomech.* 1995; 28:1451–1457. [PubMed: 8666585]
525. Shyy JY, Lin MC, Han J, Lu Y, Petrime M, Chien S. The *cis*-acting phorbol ester “12-*O*-tetradecanoylphorbol 13-acetate”-responsive element is involved in shear stress-induced monocyte chemoattractant protein 1 gene expression. *Proc Natl Acad Sci USA.* 1995; 92:8069–8073. [PubMed: 7644539]

526. Silacci P, Desgeorges A, Mazzolai L, Chambaz C, Hayoz D. Flow pulsatility is a critical determinant of oxidative stress in endothelial cells. *Hypertension*. 2001; 38:1162–1166. [PubMed: 11711515]
527. Silacci P, Formentin K, Bouzourene K, Daniel F, Brunner HR, Hayoz D. Unidirectional and oscillatory shear stress differentially modulate NOS III gene expression. *Nitric Oxide*. 2000; 4:47–56. [PubMed: 10733872]
528. Simmonds J, Fenton M, Dewar C, Ellins E, Storry C, Cubitt D, Deanfield J, Klein N, Halcox J, Burch M. Endothelial dysfunction and cytomegalovirus replication in pediatric heart transplantation. *Circulation*. 2008; 117:2657–2661. [PubMed: 18474812]
529. Simmons CA, Grant GR, Manduchi E, Davies PF. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. *Circ Res*. 2005; 96:792–799. [PubMed: 15761200]
530. Singh RN. Flow disturbance due to venous valves: a cause of graft failure. *Cathet Cardiovasc Diagn*. 1986; 12:35–38. [PubMed: 3485473]
531. Singh RN, Sosa JA, Green GE. Internal mammary artery versus saphenous vein graft. Comparative performance in patients with combined revascularization. *Br Heart J*. 1983; 50:48–58. [PubMed: 6602619]
532. Skilbeck C, Westwood SM, Walker PG, David T, Nash GB. Dependence of adhesive behavior of neutrophils on local fluid dynamics in a region with recirculating flow. *Biorheology*. 2001; 38:213–227. [PubMed: 11381176]
533. Slysh S, Goldberg S, Dervan JP, Zalewski A. Unstable angina and evolving myocardial infarction following coronary bypass surgery: pathogenesis and treatment with interventional catheterization. *Am Heart J*. 1985; 109:744–752. [PubMed: 3157302]
534. Smedby O, Hogman N, Bergstrand L. Development of femoral atherosclerosis in relation to flow disturbances. *J Biomech*. 1996; 29:543–547. [PubMed: 8964784]
535. Smedby O, Hogman N, Nilsson S, Erikson U. Flow disturbances in early femoral atherosclerosis: an in vivo study with digitized cineangiography. *J Biomech*. 1993; 26:1105–1115. [PubMed: 8408092]
536. Sorescu GP, Song H, Tressel SL, Hwang J, Dikalov S, Smith DA, Boyd NL, Platt MO, Lassègue B, Griendling KK, Jo H. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress induces monocyte adhesion by stimulating reactive oxygen species production from a nox1-based NADPH oxidase. *Circ Res*. 2004; 95:773–779. [PubMed: 15388638]
537. Sorescu GP, Sykes M, Weiss D, Platt MO, Saha A, Hwang J, Boyd N, Boo YC, Vega JD, Taylor WR, Jo H. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress stimulates an inflammatory response. *J Biol Chem*. 2003; 278:31128–31135. [PubMed: 12766166]
538. St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B, Kinzler KW. Genes expressed in human tumor endothelium. *Science*. 2000; 289:1197–1202. [PubMed: 10947988]
539. Staalsen NH, Ulrich M, Winther J, Pedersen EM, How T, Nygaard H. The anastomosis angle does change the flow fields at vascular end-to-side anastomoses in vivo. *J Vasc Surg*. 1995; 21:460–471. [PubMed: 7877228]
540. Staughton TJ, Lever MJ, Weinberg PD. Effect of altered flow on the pattern of permeability around rabbit aortic branches. *Am J Physiol Heart Circ Physiol*. 2001; 281:H53–H59. [PubMed: 11406468]
541. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med*. 2002; 8:1211–1217. [PubMed: 12411947]
542. Stemeran MB, Morrel EM, Burke KR, Colton CK, Smith KA, Lees RS. Local variation in arterial wall permeability to low density lipoprotein in normal rabbit aorta. *Arteriosclerosis*. 1986; 6:64–69. [PubMed: 3942560]
543. Stone PH, Coskun AU, Kinlay S, Clark ME, Sonka M, Wahle A, Ilegbusi OJ, Yeghiazarians Y, Popma JJ, Orav J, Kuntz RE, Feldman CL. Effect of endothelial shear stress on the progression of coronary artery disease, vascular remodeling, and in-stent restenosis in humans: in vivo 6-month follow-up study. *Circulation*. 2003; 108:438–444. [PubMed: 12860915]

544. Stone PH, Coskun AU, Kinlay S, Popma JJ, Sonka M, Wahle A, Yeghiazarians Y, Maynard C, Kuntz RE, Feldman CL. Regions of low endothelial shear stress are the sites where coronary plaque progresses and vascular remodeling occurs in humans: an in vivo serial study. *Eur Heart J*. 2007; 28:705–710. [PubMed: 17347172]
545. Stone PH, Coskun AU, Yeghiazarians Y, Kinlay S, Popma JJ, Kuntz RE, Feldman CL. Prediction of sites of coronary atherosclerosis progression: in vivo profiling of endothelial shear stress, lumen, and outer vessel wall characteristics to predict vascular behavior. *Curr Opin Cardiol*. 2003; 18:458–470. [PubMed: 14597887]
546. Stonebridge PA, Prescott RJ, Ruckley CV. Randomized trial comparing infrainguinal polytetrafluoroethylene bypass grafting with and without vein interposition cuff at the distal anastomosis. The Joint Vascular Research Group. *J Vasc Surg*. 1997; 26:543–550. [PubMed: 9357453]
547. Strong JP, Malcom GT, Oalman MC, Wissler RW. The PDAY Study: natural history, risk factors, and pathobiology. Pathobiological determinants of atherosclerosis in youth. *Ann NY Acad Sci*. 1997; 811:226–237. [PubMed: 9186600]
548. Sucusky P, Balachandran K, Elhammali A, Jo H, Yoganathan AP. Altered shear stress stimulates upregulation of endothelial VCAM-1 and ICAM-1 in a BMP-4- and TGF-beta1-dependent pathway. *Arterioscler Thromb Vasc Biol*. 2009; 29:254–260. [PubMed: 19023092]
549. Sullivan C, Hoying J. Flow-dependent remodeling in the carotid artery of fibroblast growth factor-2 knockout mice. *Arterioscler Thromb Vasc Biol*. 2002; 22:1100–1105. [PubMed: 12117723]
550. Sullivan VV, Hawley AE, Farris DM, Knipp BS, Varga AJ, Wroblewski SK, Thanapron P, Eagleton MJ, Myers DD, Fowlkes JB, Wakefield TW. Decrease in fibrin content of venous thrombi in selectin-deficient mice. *J Surg Res*. 2003; 109:1–7. [PubMed: 12591228]
551. Sun W, Li YS, Huang HD, Shyy JY, Chien S. microRNA: a master regulator of cellular processes for bioengineering systems. *Annu Rev Biomed Eng*. 2010; 12:1–27. [PubMed: 20415587]
552. Suo J, Ferrara DE, Sorescu D, Guldberg RE, Taylor WR, Giddens DP. Hemodynamic shear stresses in mouse aortas: implications for atherogenesis. *Arterioscler Thromb Vasc Biol*. 2007; 27:346–351. [PubMed: 17122449]
553. Suo J, Oshinski J, Giddens DP. Effects of wall motion and compliance on flow patterns in the ascending aorta. *J Biomech Eng*. 2003; 125:347–354. [PubMed: 12929239]
554. Surapisitchat J, Hoefen RJ, Pi X, Yoshizumi M, Yan C, Berk BC. Fluid shear stress inhibits TNF-alpha activation of JNK but not ERK1/2 or p38 in human umbilical vein endothelial cells: Inhibitory crosstalk among MAPK family members. *Proc Natl Acad Sci USA*. 2001; 98:6476–6481. [PubMed: 11353829]
555. Takase S, Bergan JJ, Schmid-Schönbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. *Ann Vasc Surg*. 2000; 14:427–435. [PubMed: 10990550]
556. Takase S, Delano FA, Lerond L, Bergan JJ, Schmid-Schönbein GW. Inflammation in chronic venous insufficiency. Is the problem insurmountable? *J Vasc Res*. 1999; 36:3–10. [PubMed: 10474045]
557. Takase S, Lerond L, Bergan JJ, Schmid-Schönbein GW. The inflammatory reaction during venous hypertension in the rat. *Microcirculation*. 2000; 7:41–52. [PubMed: 10708336]
558. Takase S, Lerond L, Bergan JJ, Schmid-Schönbein GW. Enhancement of reperfusion injury by elevation of microvascular pressures. *Am J Physiol Heart Circ Physiol*. 2002; 282:H1387–H1394. [PubMed: 11893575]
559. Takase S, Pascarella L, Bergan JJ, Schmid-Schönbein GW. Hypertension-induced venous valve remodeling. *J Vasc Surg*. 2004; 39:1329–1334. [PubMed: 15192576]
560. Takase S, Pascarella L, Lerond L, Bergan JJ, Schmid-Schönbein GW. Venous hypertension, inflammation and valve remodeling. *Eur J Vasc Endovasc Surg*. 2004; 28:484–493. [PubMed: 15465369]
561. Takase S, Schmid-Schönbein G, Bergan JJ. Leukocyte activation in patients with venous insufficiency. *J Vasc Surg*. 1999; 30:148–156. [PubMed: 10394165]

562. Tan MH, Sun Z, Opitz SL, Schmidt TE, Peters JH, George EL. Deletion of the alternatively spliced fibronectin EIIIA domain in mice reduces atherosclerosis. *Blood*. 2004; 104:11–18. [PubMed: 14976060]
563. Tanganelli P, Bianciardi G, Simoes C, Attino V, Tarabochia B, Weber G. Distribution of lipid and raised lesions in aortas of young people of different geographic origins (WHO-ISFC PBDAY Study). *World Health Organization-International Society and Federation of Cardiology Pathobiological Determinants of Atherosclerosis in Youth. Arterioscler Thromb*. 1993; 13:1700–1710. [PubMed: 8218111]
564. Tardy Y, Resnick N, Nagel T, Gimbrone MA Jr, Dewey CF. Shear stress gradients remodel endothelial monolayers in vitro via a cell proliferation-migration-loss cycle. *Arterioscler Thromb Vasc Biol*. 1997; 17:3102–3106. [PubMed: 9409299]
565. Thijssen DH, Dawson EA, Tinken TM, Cable NT, Green DJ. Retrograde flow and shear rate acutely impair endothelial function in humans. *Hypertension*. 2009; 53:986–992. [PubMed: 19380611]
566. Thomas JA, Deaton RA, Hastings NE, Shang Y, Moehle CW, Eriksson U, Topouzis S, Wamhoff BR, Blackman BR, Owens GK. PDGF-DD, a novel mediator of smooth muscle cell phenotypic modulation, is upregulated in endothelial cells exposed to atherosclerosis-prone flow patterns. *Am J Physiol Heart Circ Physiol*. 2009; 296:H442–H452. [PubMed: 19028801]
567. Thorin E, Shatos MA, Shreeve SM, Walters CL, Bevan JA. Human vascular endothelium heterogeneity. A comparative study of cerebral and peripheral cultured vascular endothelial cells. *Stroke*. 1997; 28:375–381. [PubMed: 9040693]
568. Thury A, Wentzel JJ, Vinke RV, Gijzen FJ, Schuurbiens JC, Krams R, de Feyter PJ, Serruys PW, Slager CJ. Images in cardiovascular medicine. Focal in-stent restenosis near step-up: roles of low and oscillating shear stress? *Circulation*. 2002; 105:e185–e187. [PubMed: 12057999]
569. Topol EJ, Serruys PW. *Frontiers in interventional cardiology*. *Circulation*. 1998; 98:1802–1820. [PubMed: 9788837]
570. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci USA*. 1996; 93:10417–10422. [PubMed: 8816815]
571. Topper JN, Gimbrone MA Jr. Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. *Mol Med Today*. 1999; 5:40–46. [PubMed: 10088131]
572. Touyz R. Mitochondrial redox control of matrix metalloproteinase signaling in resistance arteries. *Arterioscler Thromb Vasc Biol*. 2006; 26:685–688. [PubMed: 16556862]
573. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol*. 1998; 18:677–685. [PubMed: 9598824]
574. Tressel SL, Huang RP, Tomsen N, Jo H. Laminar shear inhibits tubule formation and migration of endothelial cells by an angiopoietin-2 dependent mechanism. *Arterioscler Thromb Vasc Biol*. 2007; 27:2150–2156. [PubMed: 17673702]
575. Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A. Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol*. 1996; 16:1256–1262. [PubMed: 8857922]
576. Truskey GA, Barber KM, Robey TC, Olivier LA, Combs MP. Characterization of a sudden expansion flow chamber to study the response of endothelium to flow recirculation. *J Biomech Eng*. 1995; 117:203–210. [PubMed: 7666657]
577. Tsai MC, Chen L, Zhou J, Tang Z, Hsu TF, Wang Y, Shih YT, Peng HH, Wang N, Guan Y, Chien S, Chiu JJ. Shear stress induces synthetic-to-contractile phenotypic modulation in smooth muscle cells via PPAR- / activations by prostacyclin released by sheared endothelial cells. *Circ Res*. 2009; 105:471–480. [PubMed: 19628794]
578. Tsai YC, Hsieh HJ, Liao F, Ni CW, Chao YJ, Hsieh CY, Wang DL. Laminar flow attenuates interferon-induced inflammatory responses in endothelial cells. *Cardiovasc Res*. 2007; 74:497–505. [PubMed: 17383622]

579. Tsao PS, Buitrago R, Chan JR, Cooke JP. Fluid flow inhibits endothelial adhesiveness. Nitric oxide and transcriptional regulation of VCAM-1. *Circulation*. 1996; 94:1682–1689. [PubMed: 8840861]
580. Tulis DA, Unthank JL, Prewitt RL. Flow-induced arterial remodeling in rat mesenteric vasculature. *Am J Physiol Heart Circ Physiol*. 1998; 274:H874–H882.
581. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*. 2005; 437:426–431. [PubMed: 16163360]
582. Uematsu M, Ohara Y, Navas JP, Nishida K, Murphy TJ, Alexander RW, Nerem RM, Harrison DG. Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. *Am J Physiol Cell Physiol*. 1995; 269:C1371–C1378.
583. Urbich C, Fritzenwanger M, Zeiher AM, Dimmeler S. Laminar shear stress upregulates the complement-inhibitory protein clusterin: a novel potent defense mechanism against complement-induced endothelial cell activation. *Circulation*. 2000; 101:352–355. [PubMed: 10653823]
584. Usami S, Chen HH, Zhao Y, Chien S, Skalak R. Design and construction of a linear shear stress flow chamber. *Ann Biomed Eng*. 1993; 21:77–83. [PubMed: 8434823]
585. Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. *Genes Dev*. 2006; 20:515–524. [PubMed: 16510870]
586. Van den Berg BM, Spaan JA, Rolf TM, Vink H. Atherogenic region and diet diminish glycocalyx dimension and increase intima-to-media ratios at murine carotid artery bifurcation. *Am J Physiol Heart Circ Physiol*. 2006; 290:H915–H920. [PubMed: 16155109]
587. Van den Berg BM, Spaan JA, Vink H. Impaired glycocalyx barrier properties contribute to enhanced intimal low-density lipoprotein accumulation at the carotid artery bifurcation in mice. *Pflügers Arch*. 2009; 457:1199–1206. [PubMed: 18839207]
588. Van der Heiden K, Groenendijk BC, Hierck BP, Hogers B, Koerten HK, Mommaas AM, Gittenberger-de Groot AC, Poelmann RE. Monocilia on chicken embryonic endocardium in low shear stress areas. *Dev Dyn*. 2006; 235:19–28. [PubMed: 16145662]
589. Van der Heiden K, Hierck BP, Krams R, de Crom R, Cheng C, Baiker M, Pourquie MJ, Alkemade FE, DeRuiter MC, Gittenberger-de Groot AC, Poelmann RE. Endothelial primary cilia in areas of disturbed flow are at the base of atherosclerosis. *Atherosclerosis*. 2008; 196:542–550. [PubMed: 17631294]
590. VanderLaan PA, Reardon CA, Getz GS. Site specificity of atherosclerosis: site-selective responses to atherosclerotic modulators. *Arterioscler Thromb Vasc Biol*. 2004; 24:12–22. [PubMed: 14604830]
591. Virchow R. Der ateromatose prozess der arterien. *Wien Med Wochenshr*. 1856; 6:825–841.
592. Vyalov S, Langille BL, Gotlieb AI. Decreased blood flow rate disrupts endothelial repair in vivo. *Am J Pathol*. 1996; 149:2107–2118. [PubMed: 8952543]
593. Wakefield TW, Strieter RM, Schaub R, Myers DD, Prince MR, Wroblewski SK, Londy FJ, Kadell AM, Brown SL, Henke PK, Greenfield LJ. Venous thrombosis prophylaxis by inflammatory inhibition without anticoagulation therapy. *J Vasc Surg*. 2000; 31:309–324. [PubMed: 10664500]
594. Wakefield TW, Strieter RM, Wilke CA, Kadell AM, Wroblewski SK, Burdick MD, Schmidt R, Kunkel SL, Greenfield LJ. Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. *Arterioscler Thromb Vasc Biol*. 1995; 15:258–268. [PubMed: 7749835]
595. Walburn FJ, Sabbah HN, Stein PD. Flow visualization in a mold of an atherosclerotic human abdominal aorta. *J Biomech Eng*. 1981; 103:168–170. [PubMed: 7278194]
596. Walpole PL, Gotlieb AI, Cybulsky MI, Langille BL. Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress. *Arterioscler Thromb Vasc Biol*. 1995; 15:2–10. [PubMed: 7538423]
597. Walshe TE, D'Amore PA. The role of hypoxia in vascular injury and repair. *Annu Rev Pathol*. 2008; 3:615–643. [PubMed: 18039132]
598. Waltham M, Burnand KG, Collins M, Smith A. Vascular endothelial growth factor and basic fibroblast growth factor are found in resolving venous thrombi. *J Vasc Surg*. 2000; 32:988–996. [PubMed: 11054231]

599. Wang KC, Garmire LX, Young A, Nguyen P, Trinh A, Subramaniam S, Wang N, Shyy JY, Li YS, Chien S. Role of microRNA-23b in flow-regulation of Rb phosphorylation and endothelial cell growth. *Proc Natl Acad Sci USA*. 2010; 107:3234–3239. [PubMed: 20133741]
600. Wang N, Miao H, Li YS, Zhang P, Haga JH, Hu Y, Young A, Yuan S, Nguyen P, Wu CC, Chien S. Shear stress regulation of Kruppel-like factor 2 expression is flow pattern-specific. *Biochem Biophys Res Commun*. 2006; 341:1244–1251. [PubMed: 16466697]
601. Wang W, Ha CH, Jhun BS, Wong C, Jain MK, Jin ZG. Fluid shear stress stimulates phosphorylation-dependent nuclear export of HDAC5 and mediates expression of KLF2 and eNOS. *Blood*. 2010; 115:2971–2979. [PubMed: 20042720]
602. Wang Y, Botvinick EL, Zhao Y, Berns MW, Usami S, Tsien RY, Chien S. Visualizing the mechanical activation of Src. *Nature*. 2005; 434:1040–1045. [PubMed: 15846350]
603. Ward MR, Pasterkamp G, Yeung AC, Borst C. Arterial remodeling. Mechanisms and clinical implications. *Circulation*. 2000; 102:1186–1191. [PubMed: 10973850]
604. Ward MR, Tsao PS, Agrotis A, Dilley RJ, Jennings GL, Bobik A. Low blood flow after angioplasty augments mechanisms of restenosis: inward vessel remodeling, cell migration, and activity of genes regulating migration. *Arterioscler Thromb Vasc Biol*. 2001; 21:208–213. [PubMed: 11156854]
605. Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, Bohm M, Meinertz T, Munzel T. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. *Circulation*. 1999; 99:2027–2033. [PubMed: 10209008]
606. Watson T, Shantsila E, Lip GY. Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited. *Lancet*. 2009; 373:155–166. [PubMed: 19135613]
607. Weinbaum S, Chien S. Lipid transport aspects of atherogenesis. *J Biomech Eng*. 1993; 115:602–610. [PubMed: 8302048]
608. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng*. 2007; 9:121–167. [PubMed: 17373886]
609. Weinbaum S, Tzenghai G, Ganatos P, Pfeffer R, Chien S. Effect of cell turnover and leaky junctions on arterial macromolecular transport. *Am J Physiol Heart Circ Physiol*. 1985; 248:H945–H960.
610. Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC. Mechanotransduction and flow across the endothelial glycocalyx. *Proc Natl Acad Sci USA*. 2003; 100:7988–7995. [PubMed: 12810946]
611. Weinberg PD. Rate-limiting steps in the development of atherosclerosis: the response-to-influx theory. *J Vasc Res*. 2004; 41:1–17. [PubMed: 14726628]
612. Weis M, von Scheidt W. Cardiac allograft vasculopathy: a review. *Circulation*. 1997; 96:2069–2077. [PubMed: 9323100]
613. Wensing PJ, Meiss L, Mali WP, Hillen B. Early atherosclerotic lesions spiraling through the femoral artery. *Arterioscler Thromb Vasc Biol*. 1998; 18:1554–1558. [PubMed: 9763526]
614. White CR, Haidekker M, Bao X, Frangos JA. Temporal gradients in shear, but not spatial gradients, stimulate endothelial cell proliferation. *Circulation*. 2001; 103:2508–2513. [PubMed: 11369693]
615. Widder JD, Chen W, Li L, Dikalov S, Thöny B, Hatakeyama K, Harrison DG. Regulation of tetrahydrobiopterin biosynthesis by shear stress. *Circ Res*. 2007; 101:830–838. [PubMed: 17704208]
616. Wilcox JN, Smith KM, Williams LT, Schwartz SM, Gordon D. Platelet-derived growth factor mRNA detection in human atherosclerotic plaques by in situ hybridization. *J Clin Invest*. 1988; 82:1134–1143. [PubMed: 2843568]
617. Wilkinson LS, Bunker C, Edwards JC, Scurr JH, Smith PD. Leukocytes: their role in the etiopathogenesis of skin damage in venous disease. *J Vasc Surg*. 1993; 17:669–675. [PubMed: 8464084]
618. Willerson JT. Systemic and local inflammation in patients with unstable atherosclerotic plaques. *Prog Cardiovasc Dis*. 2002; 44:469–478. [PubMed: 12077720]
619. Wilms H, Delano FA, Schmid-Schönbein GW. Mechanisms of parenchymal cell death in-vivo after microvascular hemorrhage. *Microcirculation*. 2000; 7:1–11. [PubMed: 10708333]

620. Wilson SH, Caplice NM, Simari RD, Holmes DR Jr, Carlson PJ, Lerman A. Activated nuclear factor-kappaB is present in the coronary vasculature in experimental hypercholesterolemia. *Atherosclerosis*. 2000; 148:23–30. [PubMed: 10580167]
621. Winnier GE, Kume T, Deng K, Rogers R, Bundy J, Raines C, Walter MA, Hogan BL, Conway SJ. Roles for the winged helix transcription factors MF1 and MFH1 in cardiovascular development revealed by nonallelic noncomplementation of null alleles. *Dev Biol*. 1999; 213:418–431. [PubMed: 10479458]
622. Wissler RW. An overview of the quantitative influence of several risk factors on progression of atherosclerosis in young people in the United States: Pathobiological Determinants of Atherosclerosis in Youth (PDAY) research group. *Am J Med Sci*. 1995; 310:S29–S36. [PubMed: 7503120]
623. Wissler RW, Strong JP. Risk factors and progression of atherosclerosis in youth. PDAY Research Group Pathological Determinants of Atherosclerosis in Youth. *Am J Pathol*. 1998; 153:1023–1033. [PubMed: 9777934]
624. Wong LC, Langille BL. Developmental remodeling of the internal elastic lamina of rabbit arteries: effect of blood flow. *Circ Res*. 1996; 78:799–805. [PubMed: 8620599]
625. Wootton DM, Ku DN. Fluid mechanics of vascular systems, diseases, and thrombosis. *Annu Rev Biomed Eng*. 1999; 1:299–329. [PubMed: 11701491]
626. Wright HP. Mitosis patterns in aortic endothelium. *Atherosclerosis*. 1972; 15:93–100. [PubMed: 5013281]
627. Wu CH, Lin CS, Hung JS, Wu CJ, Lo PH, Jin G, Shyy YJ, Mao SJ, Chien S. Inhibition of neointimal formation in porcine coronary artery by a Ras mutant. *J Surg Res*. 2001; 99:100–106. [PubMed: 11421610]
628. Wu MH, Kouchi Y, Onuki Y, Shi Q, Yoshida H, Kaplan S, Viggers RF, Ghali R, Sauvage LR. Effect of differential shear stress on platelet aggregation, surface thrombosis, and endothelialization of bilateral carotid-femoral grafts in the dog. *J Vasc Surg*. 1995; 22:382–390. [PubMed: 7563399]
629. Wu QY, Drouet L, Carrier JL, Rothschild C, Berard M, Rouault C, Caen JP, Meyer D. Differential distribution of vWF in endothelial cells: comparison between normal pigs and pigs with vW disease. *Arteriosclerosis*. 1987; 7:47–54. [PubMed: 3545164]
630. Yamada T, Tomita S, Mori M, Sasatomi E, Suenaga E, Itoh T. Increased mast cell infiltration in varicose veins of the lower limbs: a possible role in the development of varices. *Surgery*. 1996; 119:494–497. [PubMed: 8619202]
631. Yamamoto K, de Waard V, Fearn C, Loskutoff DJ. Tissue distribution and regulation of murine von Willebrand factor gene expression in vivo. *Blood*. 1998; 92:2791–2801. [PubMed: 9763564]
632. Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J. Impaired flow-dependent control of vascular tone and remodeling in P2×4-deficient mice. *Nat Med*. 2006; 12:133–137. [PubMed: 16327800]
633. Yamawaki H, Lehoux S, Berk BC. Chronic physiological shear stress inhibits tumor necrosis factor-induced proinflammatory responses in rabbit aorta perfused ex vivo. *Circulation*. 2003; 108:1619–1625. [PubMed: 12963644]
634. Yamawaki H, Pan S, Lee RT, Berk BC. Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. *J Clin Invest*. 2005; 115:733–738. [PubMed: 15696199]
635. Yao Y, Rabodzey A, Dewey CF Jr. Glycocalyx modulates the motility and proliferative response of vascular endothelium to fluid shear stress. *Am J Physiol Heart Circ Physiol*. 2007; 293:H1023–H1030. [PubMed: 17468337]
636. Yoganathan AP, He Z, Casey Jones S. Fluid mechanics of heart valves. *Annu Rev Biomed Eng*. 2004; 6:331–362. [PubMed: 15255773]
637. Yoshisue H, Suzuki K, Kawabata A, Ohya T, Zhao H, Sakurada K, Taba Y, Sasaguri T, Sakai N, Yamashita S, Matsuzawa Y, Nojima N. Large scale isolation of non-uniform shear stress-responsive genes from cultured human endothelial cells through the preparation of a subtracted cDNA library. *Atherosclerosis*. 2002; 162:323–334. [PubMed: 11996952]

638. Young A, Wu W, Sun W, Larman HB, Wang N, Li YS, Shyy JY, Chien S, García-Cardena G. Flow activation of AMP-activated protein kinase in vascular endothelium leads to Kruppel-like factor 2 expression. *Arterioscler Thromb Vasc Biol.* 2009; 29:1902–1908. [PubMed: 19696400]
639. Yu J, Bergaya S, Murata T, Alp IF, Bauer MP, Lin MI, Drab M, Kurzchalia TV, Stan RV, Sessa WC. Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. *J Clin Invest.* 2006; 116:1284–1291. [PubMed: 16670769]
640. Yun S, Dardik A, Haga M, Yamashita A, Yamaguchi S, Koh Y, Madri JA, Sumpio BE. Transcription factor Sp1 phosphorylation induced by shear stress inhibits membrane type 1-matrix metalloproteinase expression in endothelium. *J Biol Chem.* 2002; 277:34808–34814. [PubMed: 12093818]
641. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med.* 2008; 359:1897–1908. [PubMed: 18971492]
642. Zakkar M, Chaudhury H, Sandvik G, Enesa K, Luongle A, Cuhlmann S, Mason JC, Krams R, Clark AR, Haskard DO, Evans PC. Increased endothelial mitogen-activated protein kinase phosphatase-1 expression suppresses proinflammatory activation at sites that are resistant to atherosclerosis. *Circ Res.* 2008; 103:726–732. [PubMed: 18723442]
643. Zampetaki A, Zeng L, Margariti A, Xiao Q, Li H, Zhang Z, Pepe AE, Wang G, Habibi O, deFalco E, Cockerill G, Mason JC, Hu Y, Xu Q. Histone deacetylase 3 is critical in endothelial survival and atherosclerosis development in response to disturbed flow. *Circulation.* 2010; 121:132–142. [PubMed: 20026773]
644. Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, Glagov S. Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. *Circ Res.* 1983; 53:502–514. [PubMed: 6627609]
645. Zarins CK, Zatina MA, Giddens DP, Ku DN, Glagov S. Shear stress regulation of artery lumen diameter in experimental atherogenesis. *J Vasc Surg.* 1987; 5:413–420. [PubMed: 3509594]
646. Zeiffer U, Schober A, Lietz M, Liehn EA, Erl W, Emans N, Yan ZQ, Weber C. Neointimal smooth muscle cells display a proinflammatory phenotype resulting in increased leukocyte recruitment mediated by P-selectin and chemokines. *Circ Res.* 2004; 94:776–784. [PubMed: 14963004]
647. Zeng L, Zampetaki A, Margariti A, Pepe AE, Alam S, Martin D, Xiao Q, Wang W, Jin ZG, Cockerill G, Mori K, Li YS, Hu Y, Chien S, Xu Q. Sustained activation of XBP1 splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow. *Proc Natl Acad Sci USA.* 2009; 106:8326–8331. [PubMed: 19416856]
648. Zeng L, Zhang Y, Chien S, Liu X, Shyy JY. The role of p53 deacetylation in p21Waf1 regulation by laminar flow. *J Biol Chem.* 2003; 278:24594–24599. [PubMed: 12716906]
649. Zhang H, Sunnarborg SW, McNaughton KK, Johns TG, Lee DC, Faber JE. Heparin-binding epidermal growth factor-like growth factor signaling in flow-induced arterial remodeling. *Circ Res.* 2008; 102:1275–1285. [PubMed: 18436796]
650. Zhang J, Burrige KA, Friedman MH. In vivo differences between endothelial transcriptional profiles of coronary and iliac arteries revealed by microarray analysis. *Am J Physiol Heart Circ Physiol.* 2008; 295:H1556–H1561. [PubMed: 18689496]
651. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* 1992; 258:468–471. [PubMed: 1411543]
652. Zhao Y, Chen BP, Miao H, Yuan S, Li YS, Hu Y, Rocke DM, Chien S. Improved significance test for DNA microarray data: temporal effects of shear stress on endothelial genes. *Physiol Genomics.* 2002; 12:1–11. [PubMed: 12399447]
653. Ziegler T, Bouzourene K, Harrison VJ, Brunner HR, Hayoz D. Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. *Arterioscler Thromb Vasc Biol.* 1998; 18:686–692. [PubMed: 9598825]

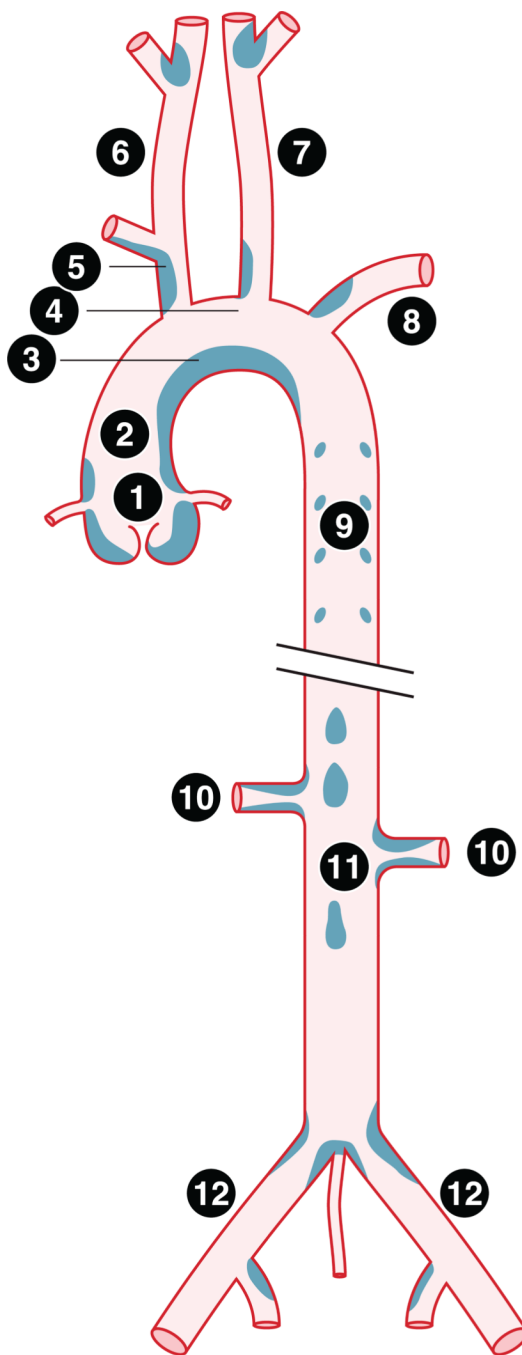


FIG. 1. Atherosclerosis preferentially develops at arterial branches and curvatures. Schematic drawing shows a longitudinal representation of the major arterial vasculature illustrating the observed distribution of atherosclerotic plaques (gray shading) in the vasculatures of $LDLR^{-/-}$ mice fed a high-fat atherogenic diet. 1, Aortic sinus; 2, ascending aorta; 3, inner (lesser) curvature of aortic arch; 4, outer (greater) curvature of aortic arch; 5, innominate artery; 6, right common carotid artery; 7, left common carotid artery; 8, left subclavian artery; 9, thoracic aorta; 10, renal artery; 11, abdominal aorta; 12, iliac artery. [Redrawn from VanderLaan et al. (590).]

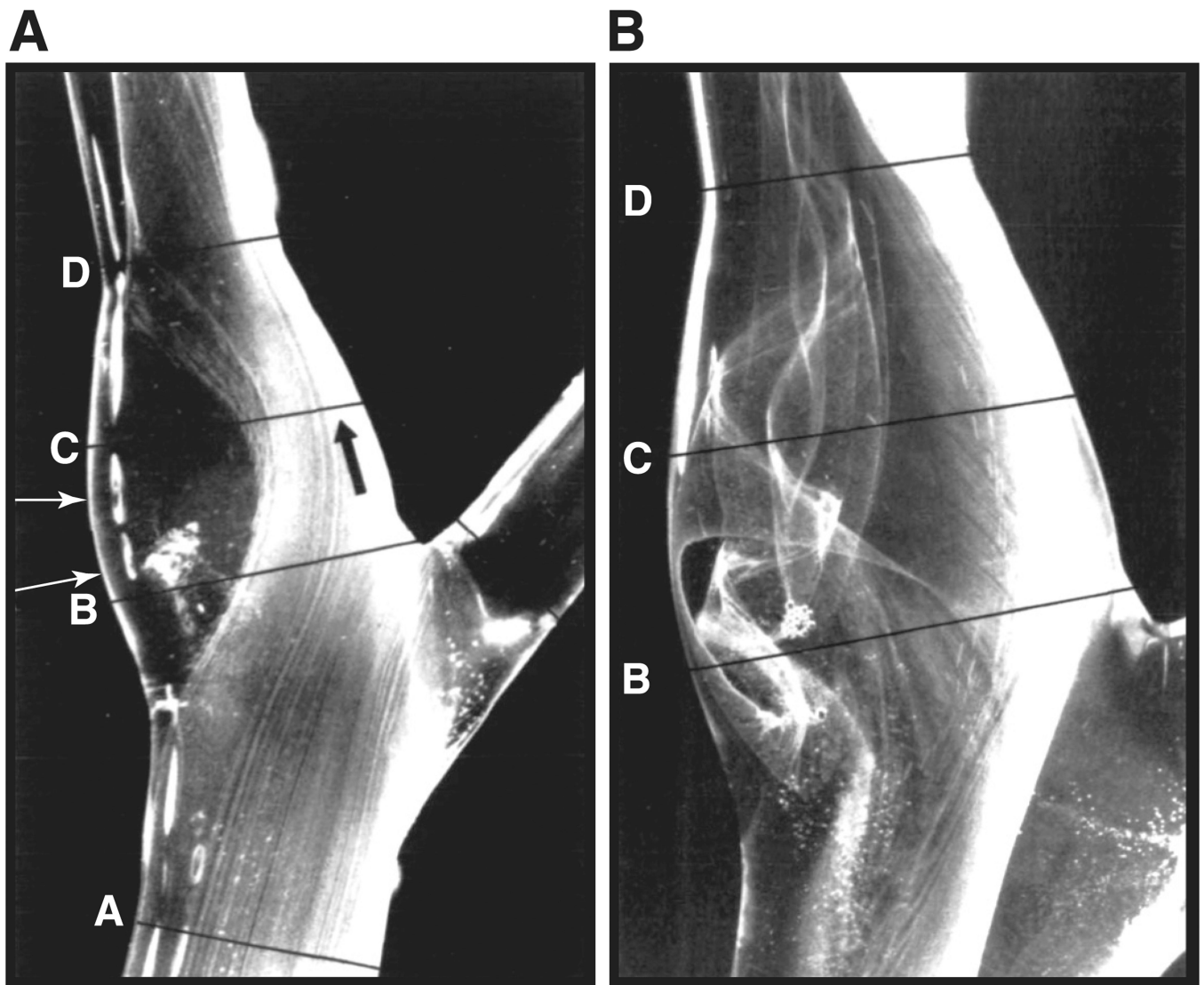


FIG. 2. Hydrogen bubble visualization of flow in molds of carotid bifurcation. Flow visualizations were made with flow division ratio between internal and external carotid branches of 70:30 and Reynolds number (based on the inlet flow rate and hydraulic diameter) of 400 (*A*) and flow division ratio of 80:20 and Reynolds number of 800 (*B*) to show the effects of these hemodynamic parameters on flow pattern. *A*: flow is rapid, laminar, and longitudinal along the common carotid artery and the inner wall of the internal carotid sinus (black arrow); a large area of flow separation is formed along the outer wall of the sinus (white arrows). *B*: streamlines are skewed towards the apex of the bifurcation, and complex helical flow patterns occupy the separated flow region of the sinus. The five areas indicated are as follows: *A*, common carotid; *B*, proximal internal carotid; *C*, midpoint of carotid sinus; *D*, distal internal carotid; *E*, external carotid. [From Zarins et al. (644).]

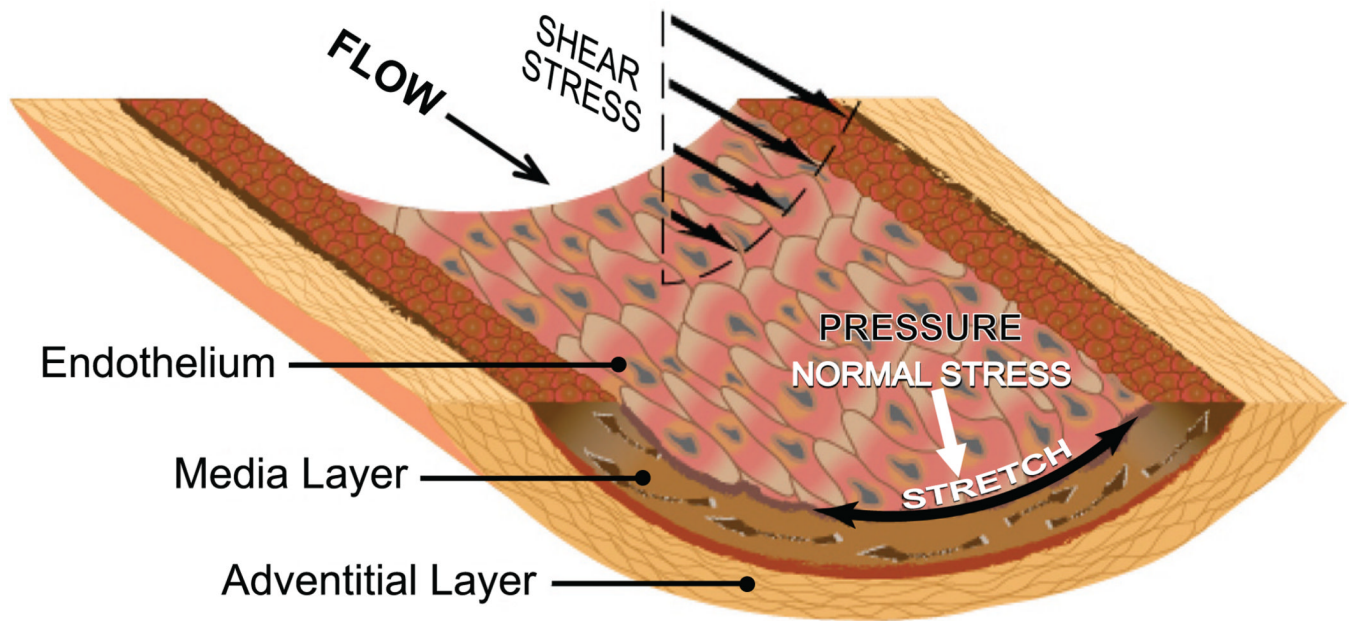


FIG. 3. Schematic diagram showing the generation of shear stress (parallel to the endothelial cell surface) by blood flow and the generation of normal stress (perpendicular to the endothelial cell surface) and circumferential stretch due to the action of pressure. (Shu Chien and Yi-Shuan Li, unpublished Figure.)

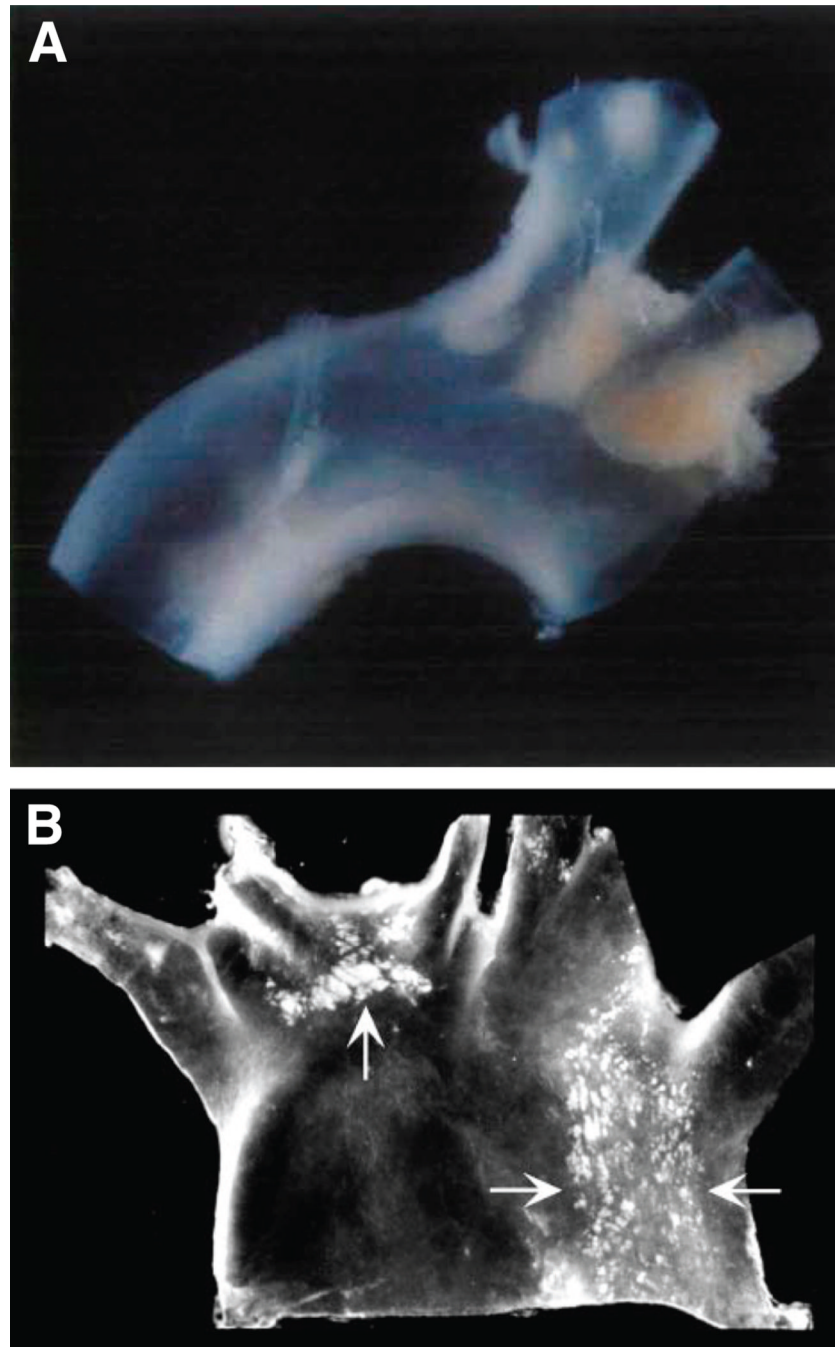


FIG. 4. Atherosclerotic lesions in the aortic arch. *A*: gross appearance of atherosclerotic lesions in the aortic arch of a 20-wk-old ApoE^{-/-} mouse fed the chow diet. Yellowish-white lesions are observed in regions of inner curvature of the aortic arch and at the orifices of the arch branches. The more yellowish material in the branches is adventitial fat tissue. *B*: thin and transparent aorta of mouse allows direct visualization of early lesions in ApoE^{-/-} mice by dissection microscopy. Under reflected light, elevated white lesions are visible on the luminal surface of the aortic arch of a 8-wk-old ApoE^{-/-} mouse fed the Western-type diet. Two areas of lesion development are prominent and contain opaque sites of lesion

formation: the lesser curvature (on the *right*, between the arrows) and the orifice of the arch branches (on the *left*, single arrow). [From Nakashima et al. (406, 407).]

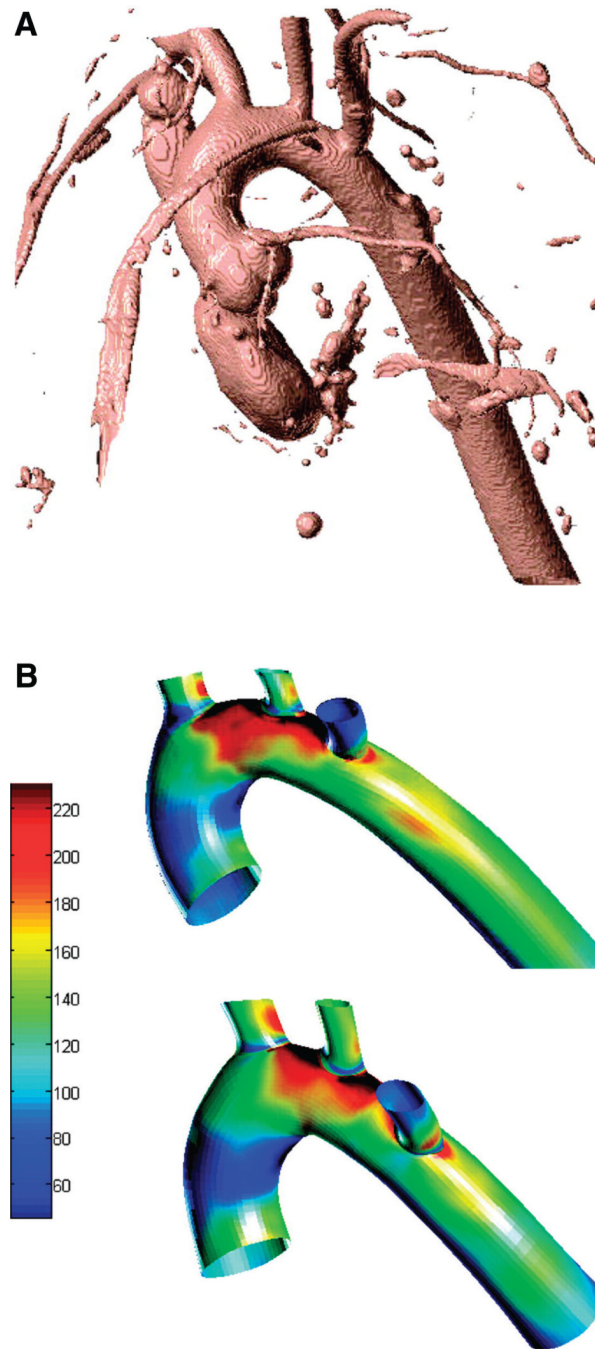
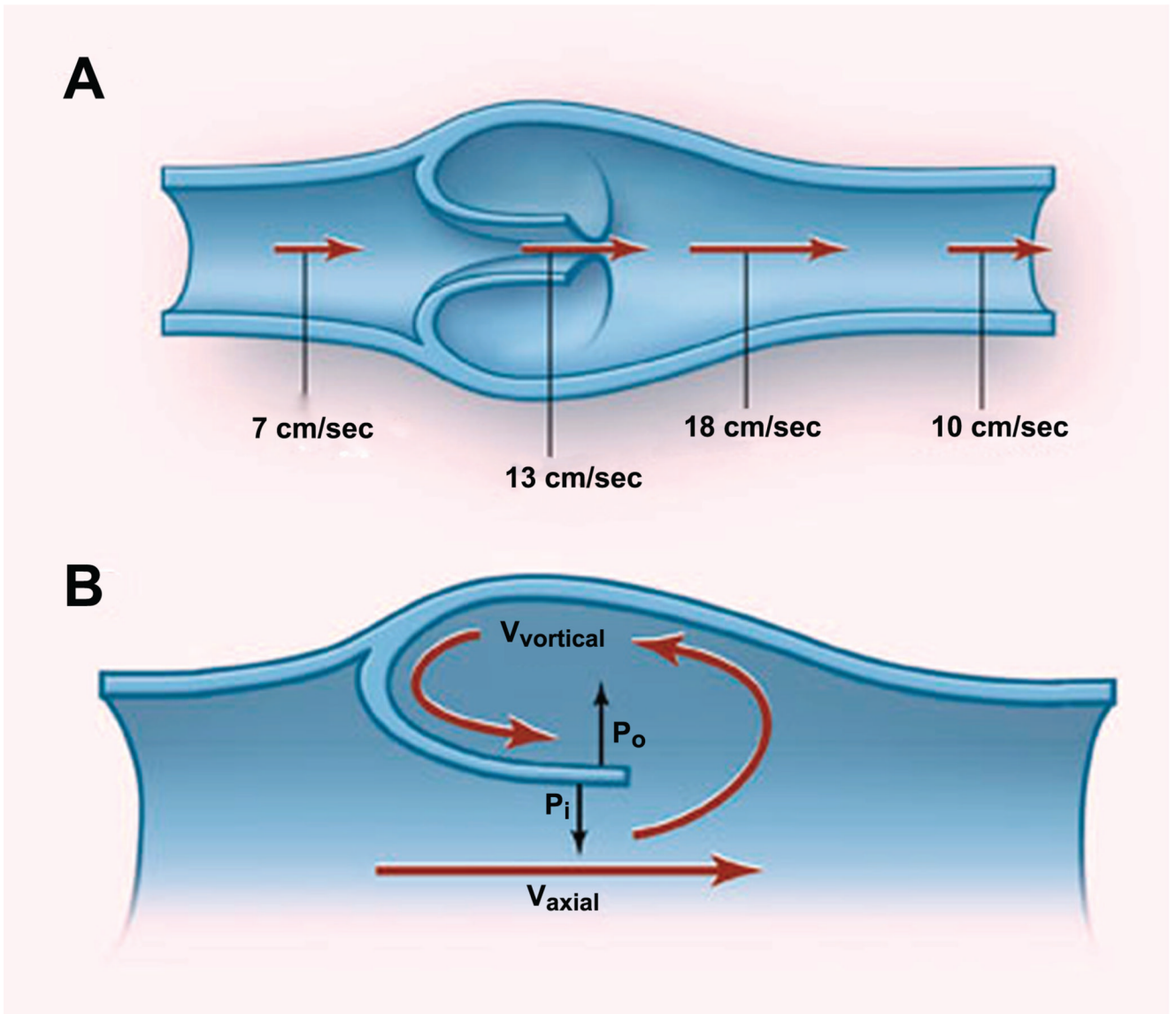


FIG. 5. Shear stress distributions in mouse aortas. *A*: a representative image of mouse aortas illustrated by micro-CT scan. For reference, the diameter of the descending aorta is ~ 1 mm. *B*: distributions of mean shear stress in the aortas. Velocities in the aortas are measured by Doppler ultrasound. Mean values of wall shear stress are computed by averaging wall shear stress magnitudes over the cardiac cycle. Colors are used for scaling the values in dyn/cm^2 , and data are shown for two different mice. The mean wall shear stress in the mouse is much higher than that in the human (553), although the inner curvature of the arch and the entrance to the orifice of the innominate artery are areas of lower shear stresses. On the other

hand, lateral surfaces of the ascending aorta and the region of the arch around the branch of left common carotid artery experience higher shear stress values. [From Suo et al. (552).]

**FIG. 6.**

Velocity of blood flow through a venous valve and forces acting on a venous valvular leaflet. *A*: the reduced cross-sectional area between the valvular leaflets produces a proximally directed jet of increased axial velocity. *B*: axial flow between the leaflets generates a pressure (P_o) that tends to keep the leaflet in the open position, and vortical flow in the valve pocket generates a pressure (P_i) that tends to close the leaflet. These pressures depend on the respective flow velocities (V_{vortical} and V_{axial}); pressure is inversely related to velocity. [From Bergan et al. (36).]

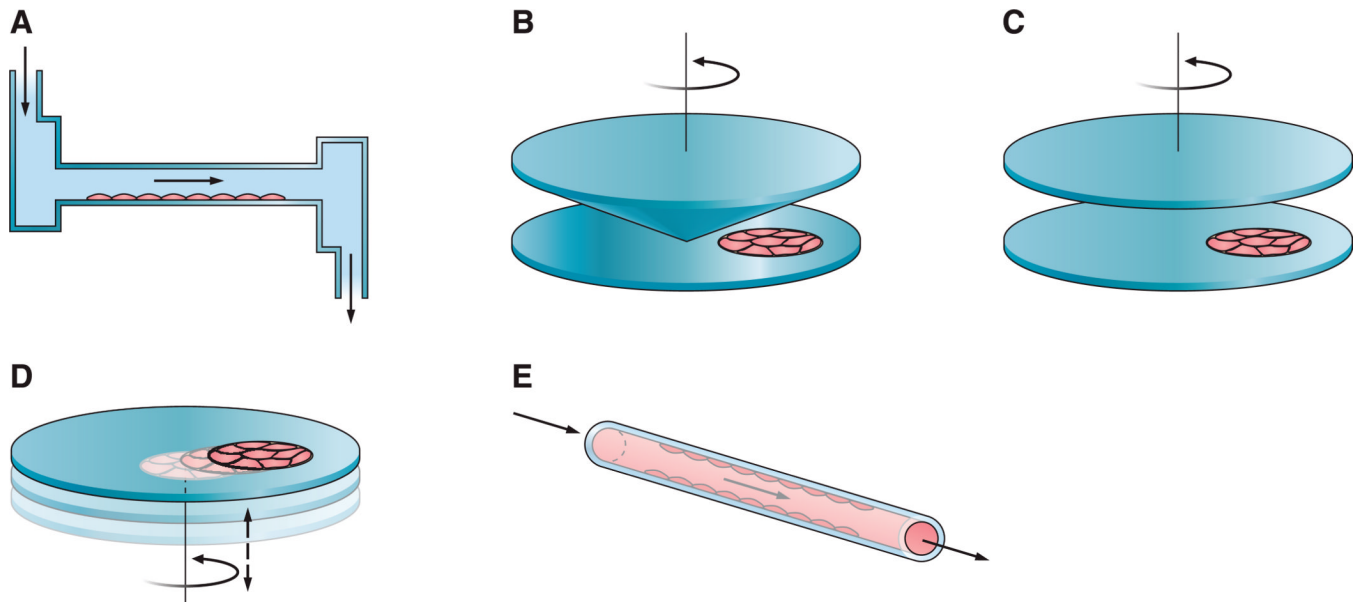


FIG. 7. The configurations for in vitro systems for applying shear flow to ECs. *A:* parallel-plate flow chamber. *B:* cone-and-plate viscometer. *C:* parallel disk viscometer. *D:* orbital shaker. *E:* tubular or rectangular capillary tube. It is noted that, for ease of visualization, the ECs are not drawn all around the inner lumen of the tube.

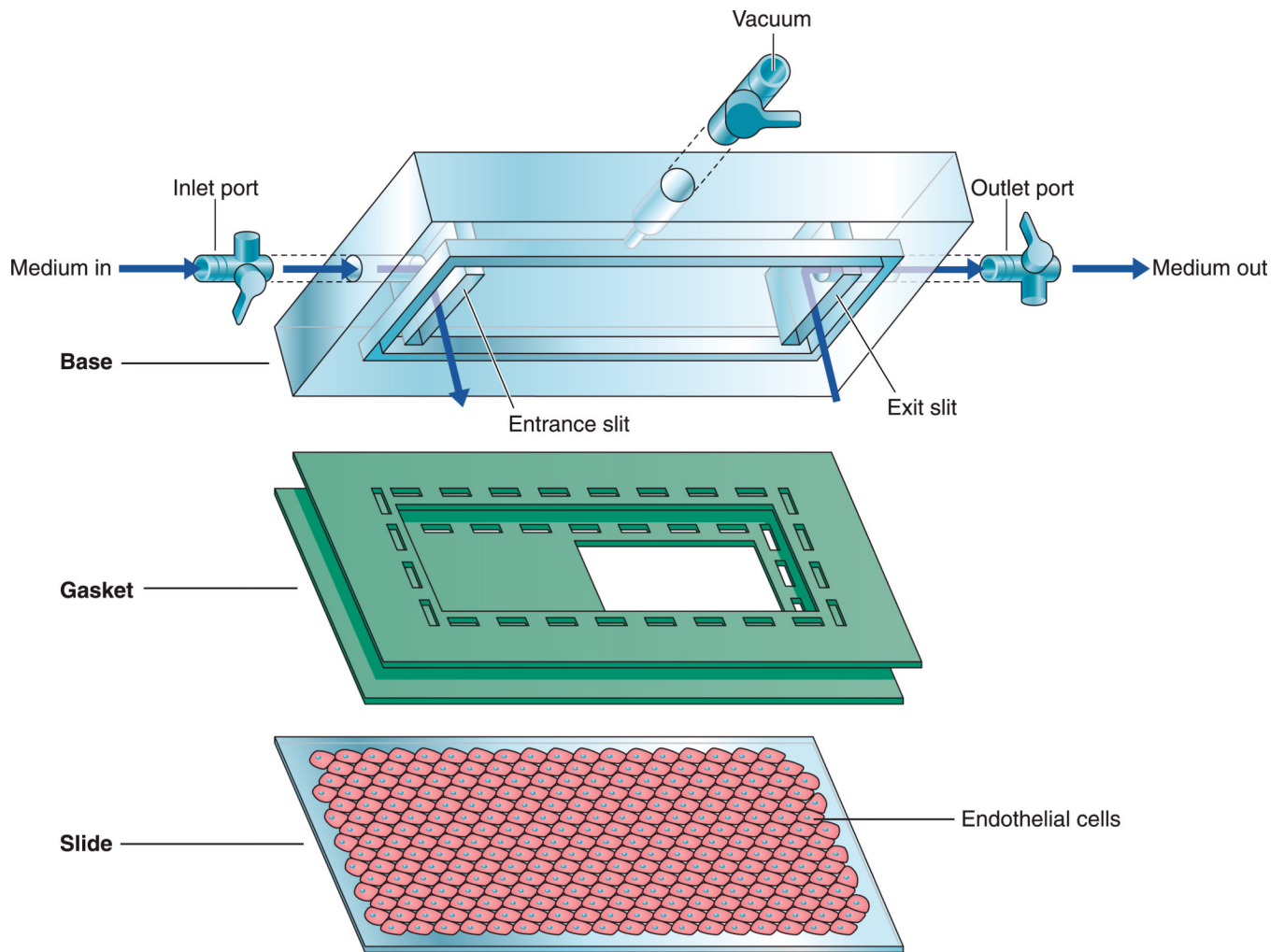


FIG. 8. Diagram showing the parallel-plate flow chamber for vertical-step flow. The polycarbonate base plate (*top*), two gaskets with different open areas, and the glass slide with EC monolayer (*bottom*) are held together by a vacuum suction applied at the perimeter of the slide, forming a channel with a lesser depth at the entrance, creating a step. Cultured medium enters at inlet port through entrance slit into the channel and exits through exit slit and outlet port.

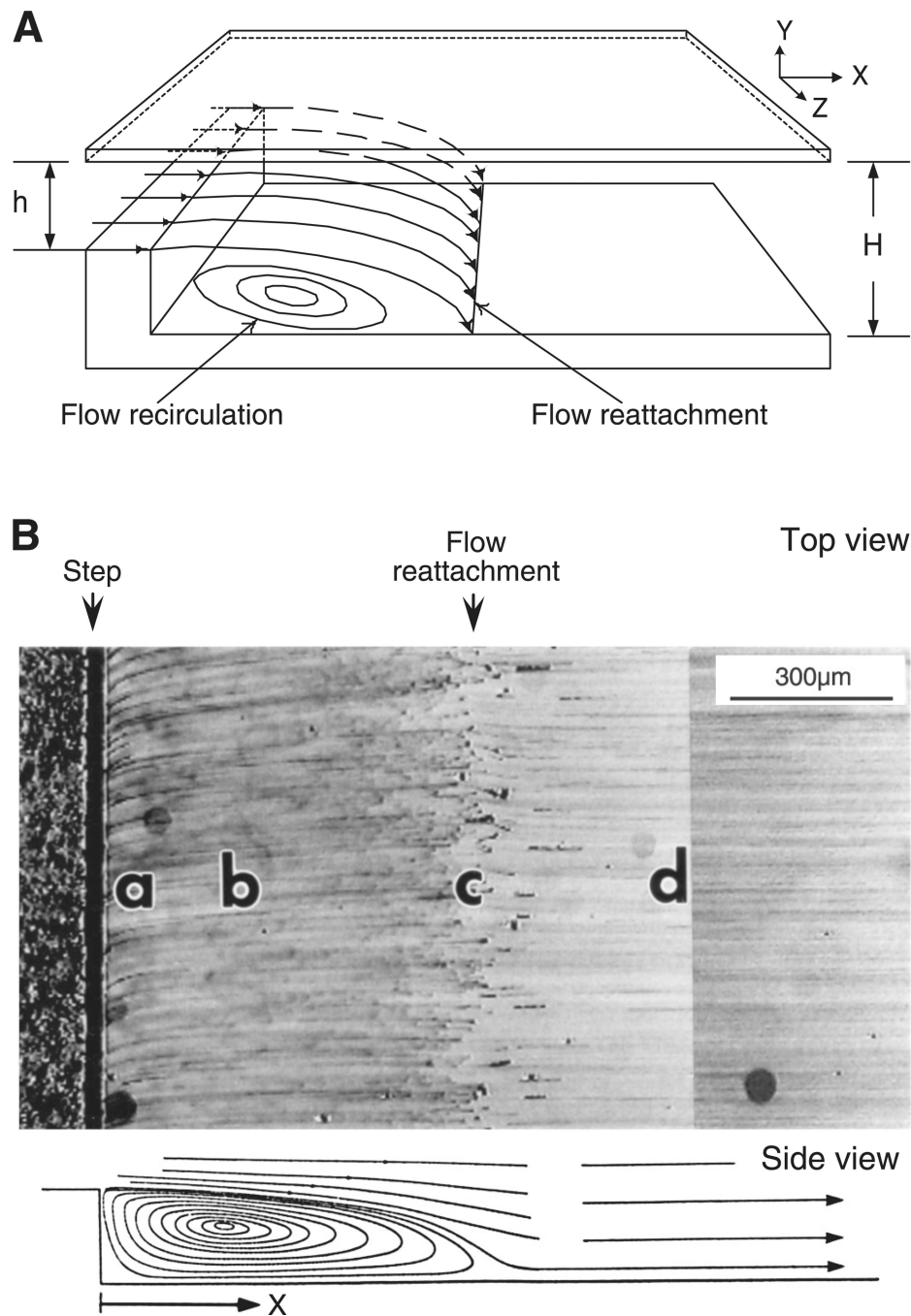


FIG. 9. Visualization of flow patterns created in vertical-step flow channel. *A*: schematic diagram of the flow channel showing the flow pattern immediately beyond the step. h and H are heights of the channel above the step and beyond the step, respectively. *B*, *top*: phase-contrast photomicrograph (top view) of experimental flow patterns in the vertical-step flow channel. Flow is from *left to right* and is made visible with marker microparticles (1 μm in diameter). Flow separation occurs in the region distal to the step, forming four specific flow areas: *a*, the stagnant flow area; *b*, the center of the recirculation eddy; *c*, the reattachment flow area; and *d*, the fully developed flow area. From on-line microscopic observations, the particles transported from the bulk flow along the curved streamlines with decreasing velocities

towards the wall near the reattachment point (area *c*). While some of the particles moved forward to rejoin the mainstream with increasing velocities, others moved in a retrograde direction towards the step as recirculation eddies. These latter particles moved upstream initially with increasing velocities and decelerated when approaching the wall of the step (area *a*), from where the particles were carried away from the floor of the chamber by upward curved streamlines. *Bottom*: schematic drawing of the side view of the streamlines in the vertical-step flow channel deduced from the top view photograph. [From Chiu et al. (88).]

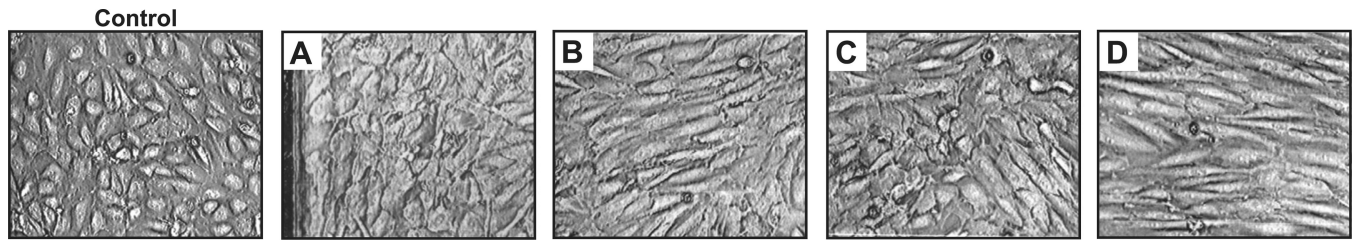


FIG. 10. Morphological changes induced in a confluent EC monolayer by exposure to disturbed flow in the step flow channel. *A*, The stagnant flow area; *B*, the center of the recirculation eddy; *C*, the reattachment flow area; *D*, the fully developed flow area (same designations as in Fig. 9). The mean values of wall shear stress created in areas *A*, *B*, *C*, and *D* are characterized to be 0.5, 20, 0, and 20 dyn/cm², respectively, by measurements using micron-resolution particle image velocimetry (μ PIV) and by computational simulation (88, 94). The direction of the main flow is *left to right*. Control photograph is from the same experimental monolayer before shearing (0 h). [Modified from Chiu et al. (94).]

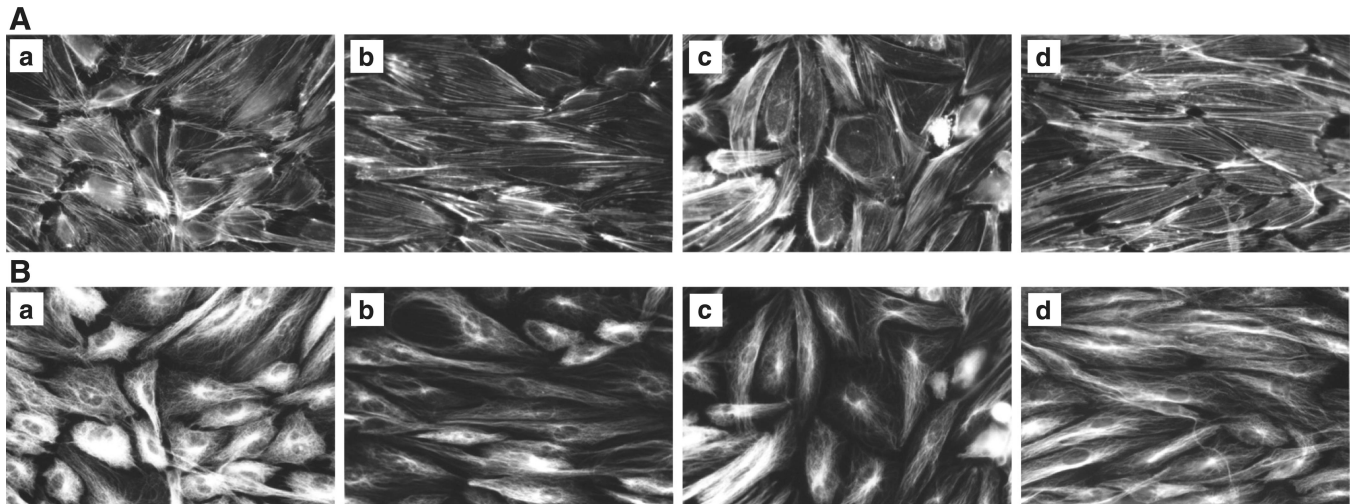


FIG. 11. Fluorescence photographs of F-actin filaments (*A*) and microtubules (*B*) after exposure to disturbed flow in the step-flow channel for 24 h. *a*, The stagnant flow area; *b*, the center of the recirculation eddy; *c*, the reattachment flow area; *d*, the fully developed flow area (same designations as in Figs. 9 and 10). The direction of the main flow is *left to right*. Magnification: $\times 400$. [Modified from Chiu et al. (94).]

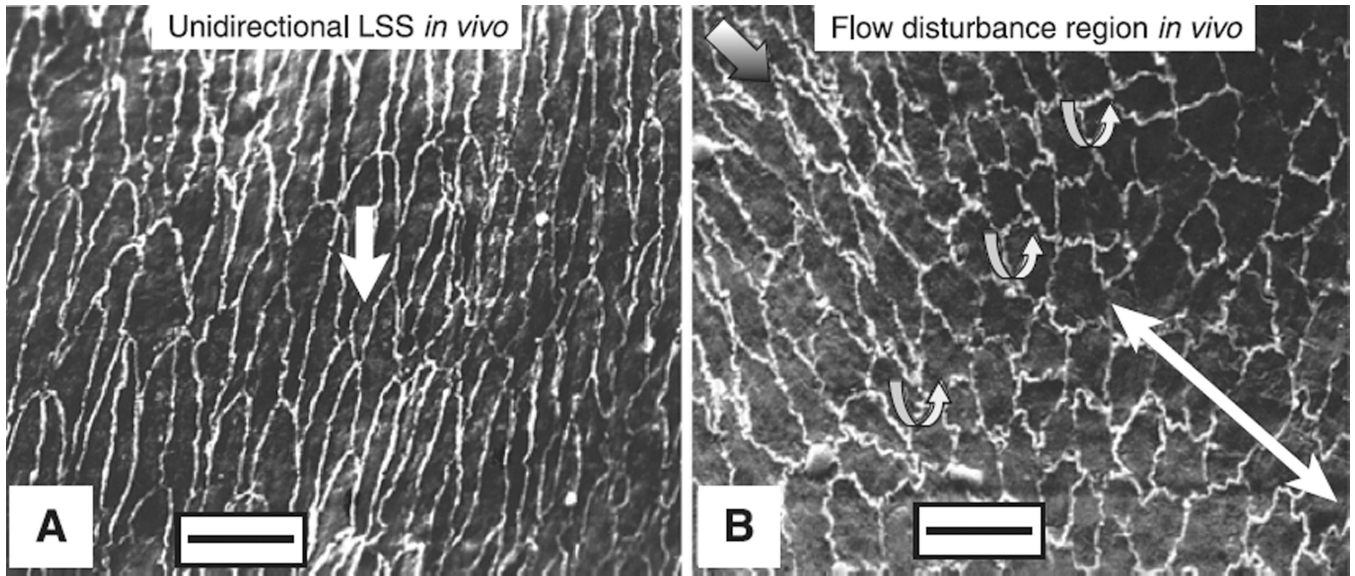


FIG. 12.

In situ observation of EC morphologies in regions of laminar and disturbed flows. *A*: EC alignment in unidirectional laminar flow in the descending thoracic aorta. The direction of flow is indicated by the downward white arrow near the center of the picture. *B*: morphological changes in ECs in regions near a branch of the aorta. ECs change their morphologies from aligned elongations to polygonal shape beyond a line of flow separation (curved arrows) that marks the boundary of a disturbed flow region where oscillating, multidirectional flow typically occurs (double-headed arrow). Scale bar in each panel = 15 μm . For details, see Davies (125). [From Davies (125).]

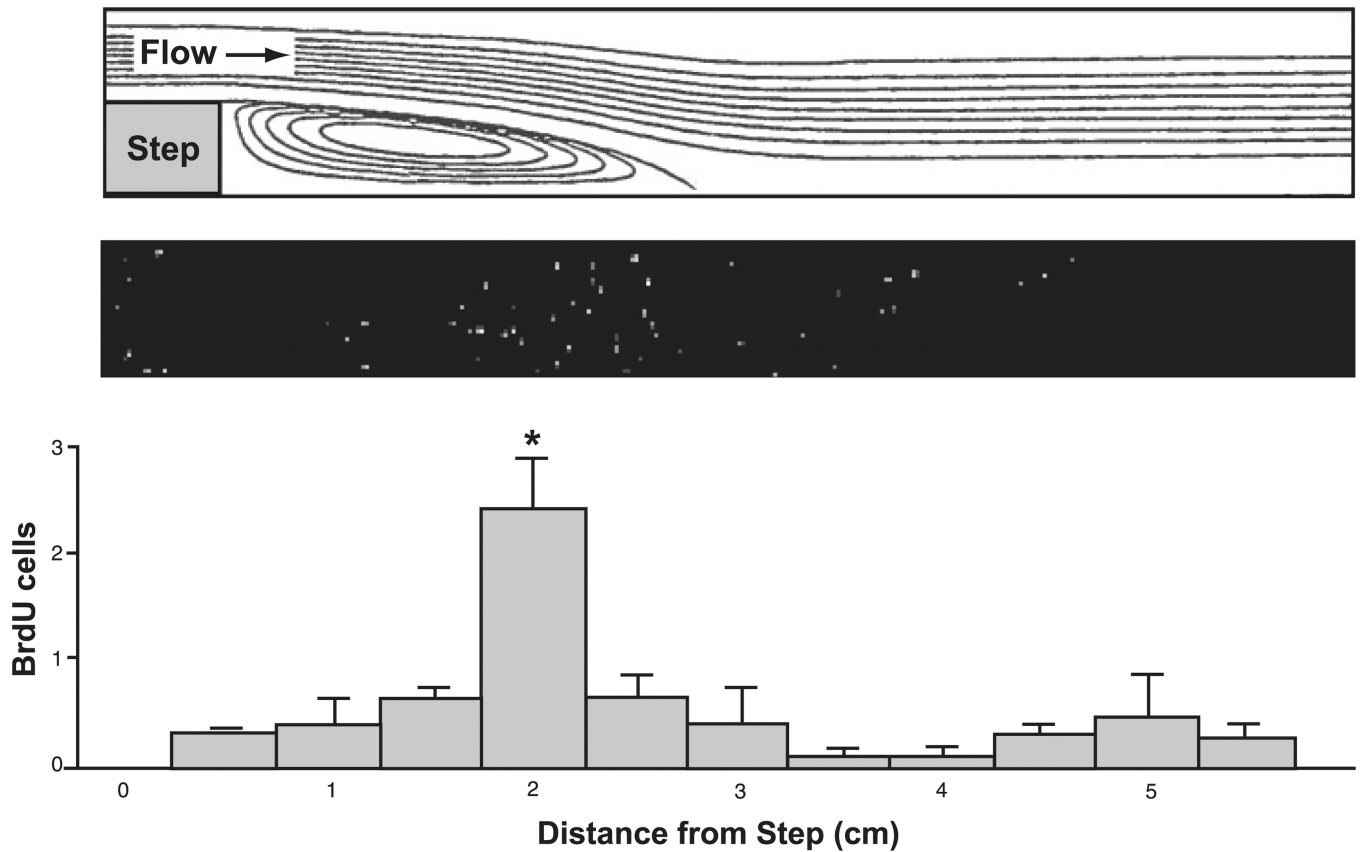
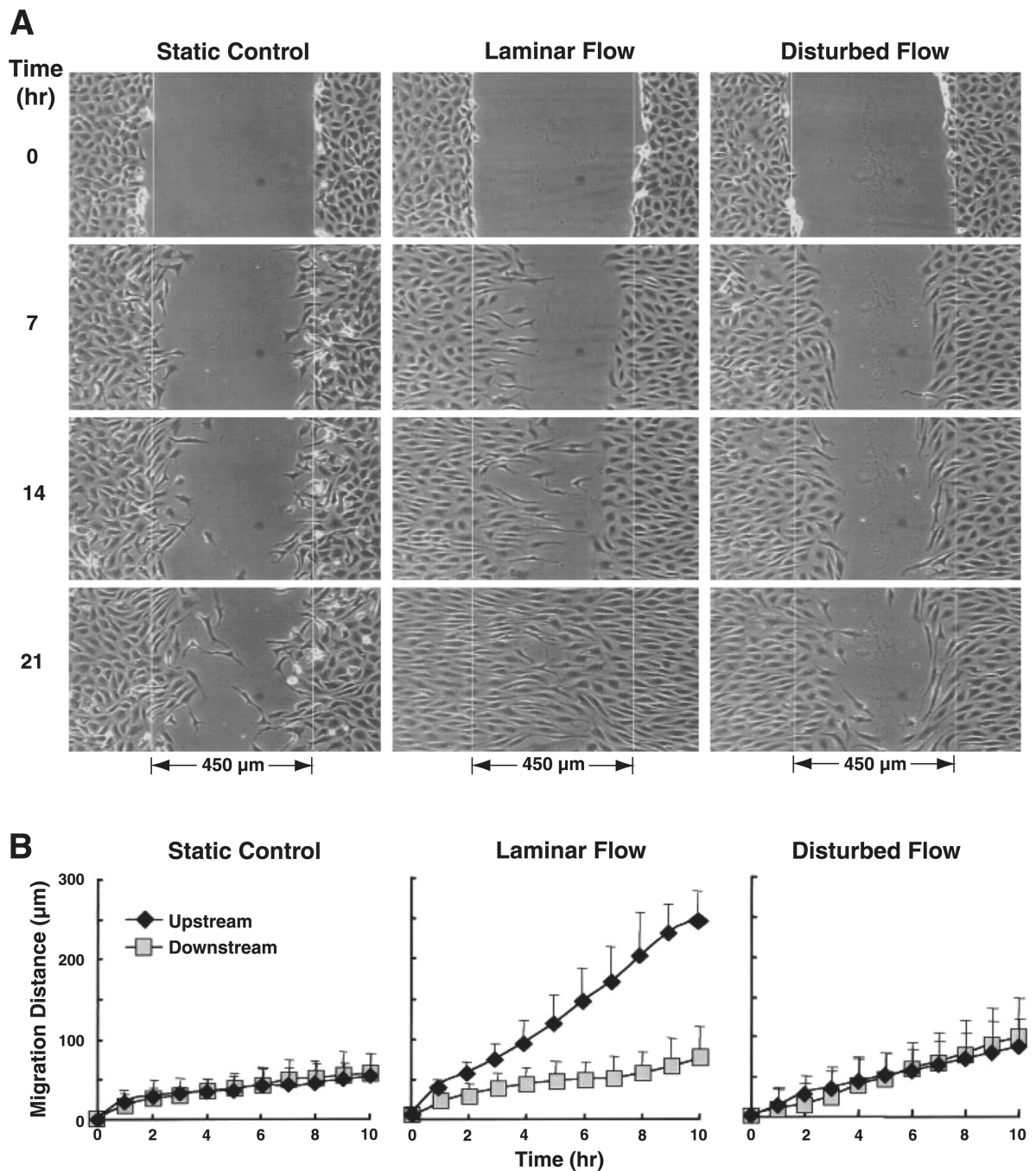


FIG. 13. EC proliferative rate is increased in regions of disturbed flow. The proliferation of cultured bovine aortic ECs, as assessed by the BrdU incorporation assay, is markedly elevated in the disturbed flow region near the reattachment point in the step-flow channel. *Top panel* shows the side view of the step flow channel. *Middle panel* shows BrdU incorporation into ECs in one experiment. *Bottom panel* shows the BrdU incorporation into the ECs in four experiments. Bars are means \pm SE. Asterisk indicates significant difference in BrdU incorporation in the region of disturbed flow near the reattachment point. Not shown in the bar graph is an increase in BrdU incorporation immediately next to the step. [From Chien (86).]

**FIG. 14.**

Laminar flow promotes EC migration compared with disturbed flow and static control. The wounded EC monolayers are kept under static conditions or are subjected to laminar or disturbed flow in a stepflow channel. The direction of laminar flow is from *left to right*. The left-hand side of the zone is denoted as “upstream” side, and the right-hand side is “downstream.” *A*: phase-contrast images of the EC migration over the 21-h period of time. White vertical lines indicate the locations of wound edges at the beginning of the experiment. *B*: the net migration distance as a function of time for the cells in the first row at wound edges (both upstream and downstream sides). [From Hsu et al. (259).]

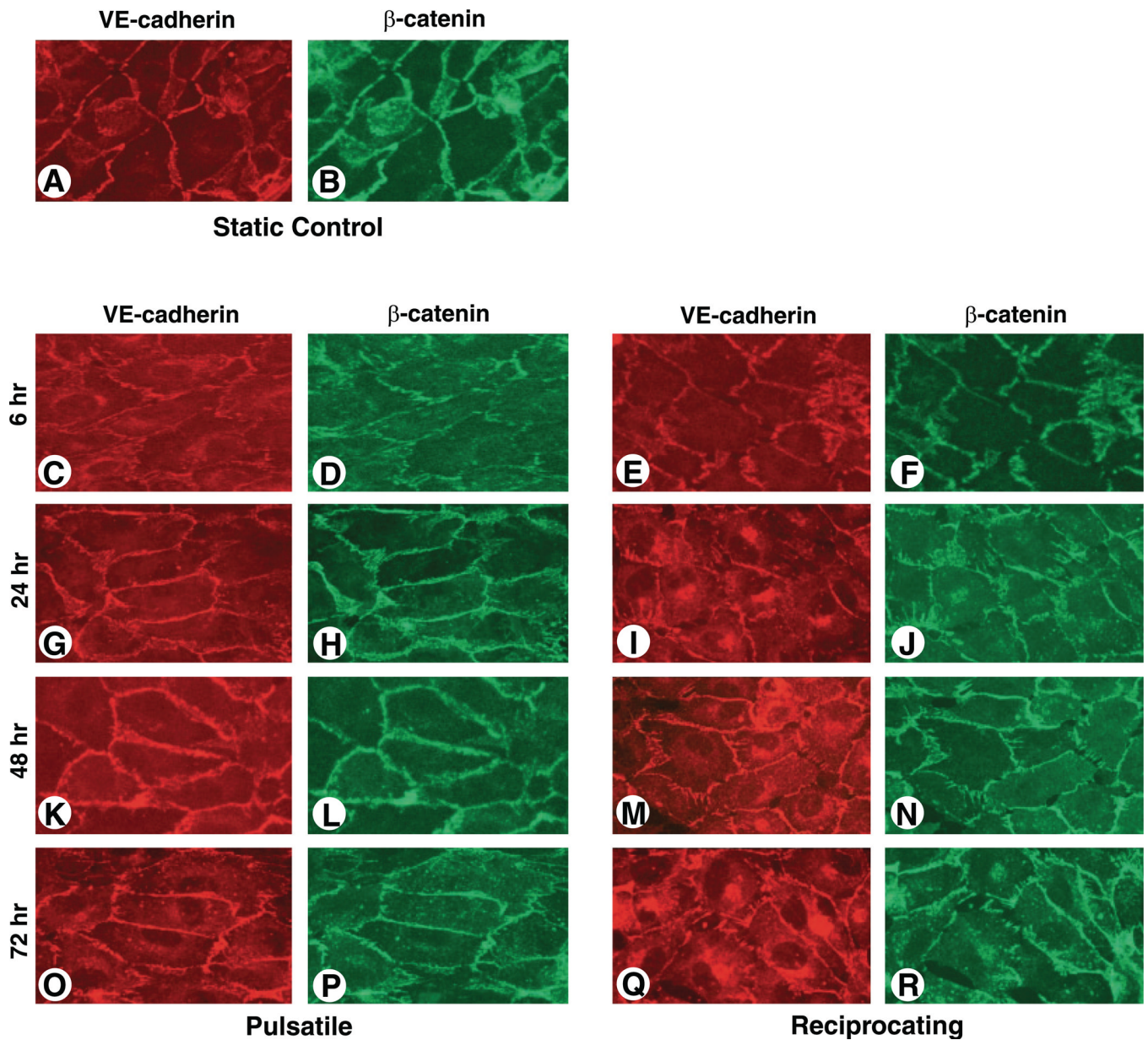


FIG. 15. VE-cadherin and β -catenin staining in ECs exposed to pulsatile and reciprocating flows. Confluent monolayers of bovine aortic ECs are kept as controls (*A, B*) or subjected to different flow patterns in a flow chamber. VE-cadherin staining is observed by confocal microscopy. Stainings for VE-cadherin and β -catenin at intercellular junctions are discontinuous after exposing to pulsatile (*C, D*) or reciprocating (*E, F*) flow for 6 h. The distribution of these junction proteins becomes continuous around the entire periphery of the cells after 24, 48, or 72 h of exposure to pulsatile flow (*G, H, K, L, O, P*), but not reciprocating flow (*I, J, M, N, Q, R*). [From Miao et al. (379).]

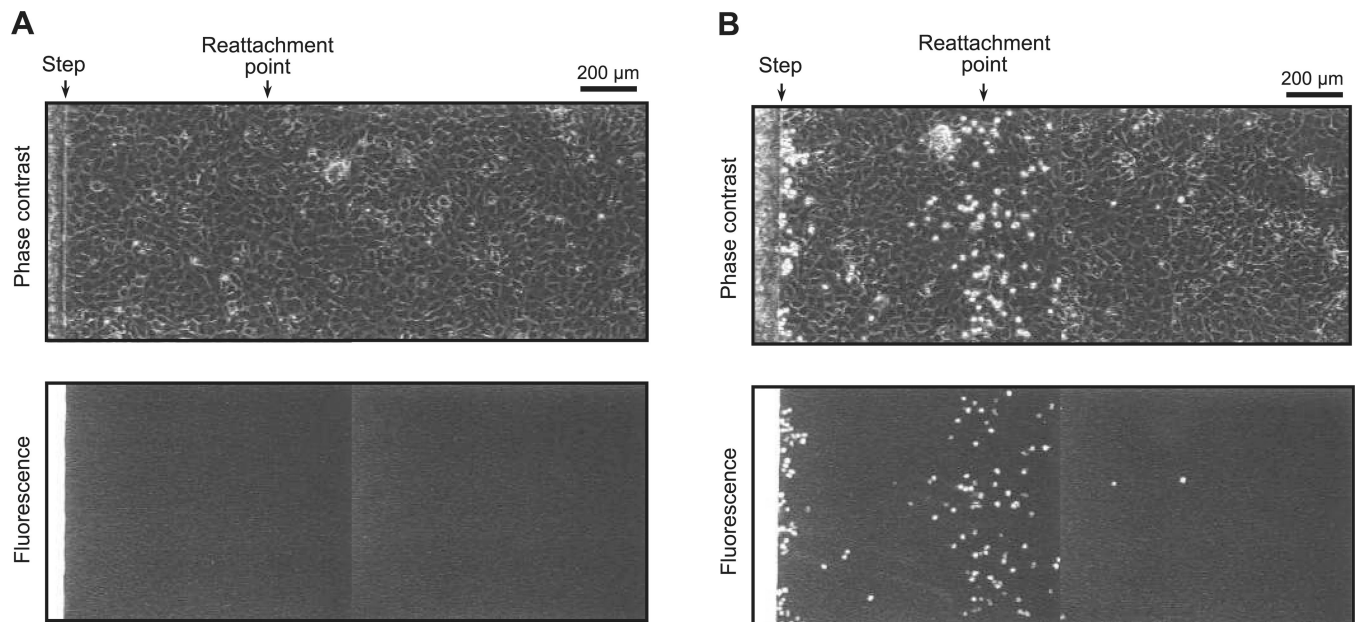


FIG. 16. Enhancement of monocytic THP-1 cell adhesion to activated ECs by disturbed flow. ECs are maintained in static condition without (A) or with TNF- α (125 U/ml) treatment (B) for 4 h, and then exposed to disturbed flow in the step-flow channel with THP-1 cell suspension at a concentration of 5×10^5 cells/ml. Prior to the perfusion, THP-1 cells are labeled with calcein-AM at a concentration of 7.5 μ M for 30 min to facilitate visualization of the cells under fluorescence optics against the endothelial background. Phase-contrast and corresponding fluorescent microphotographs are taken after 10 min of perfusion. [From Chiu et al. (88).]

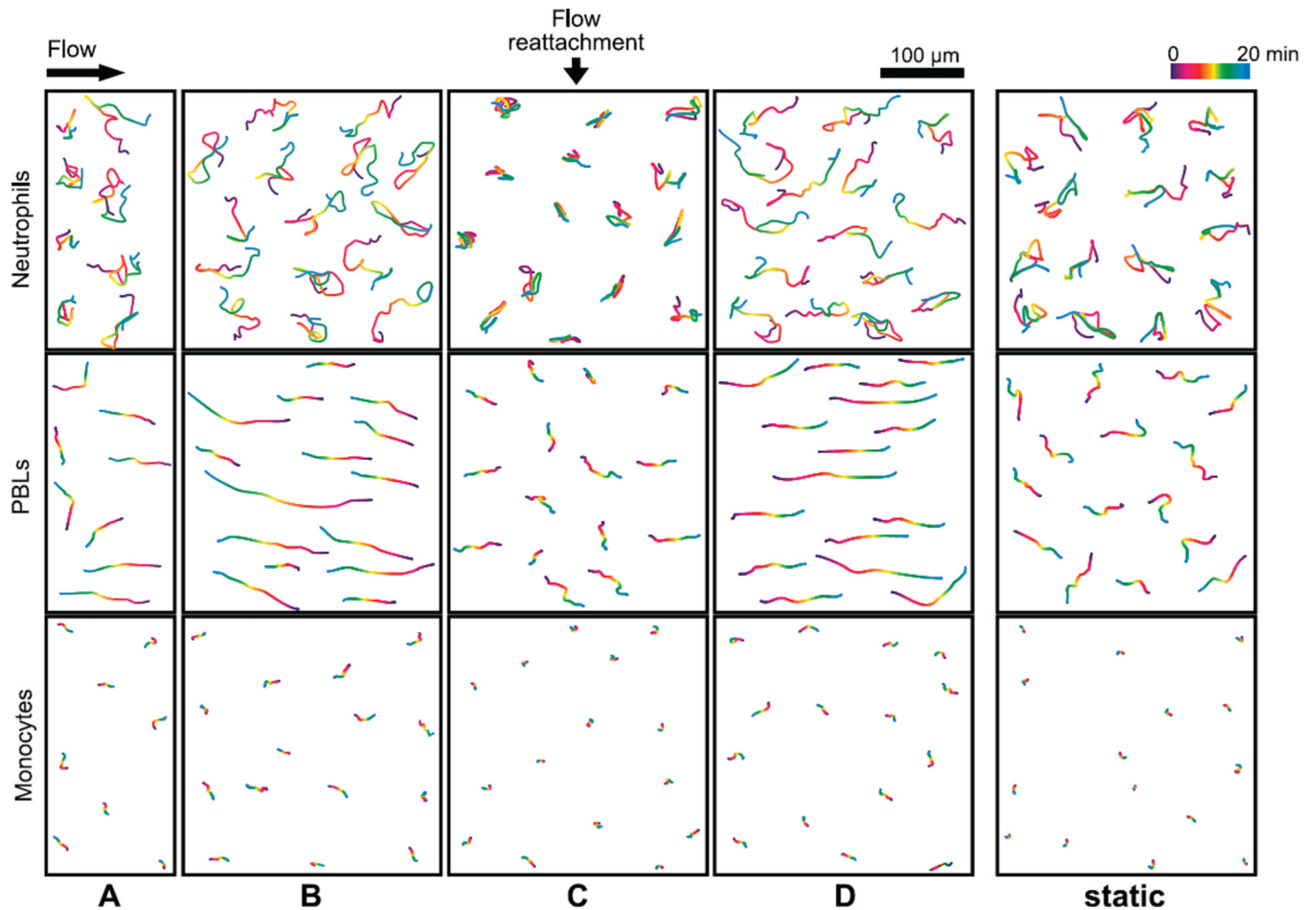


FIG. 17. Neutrophils, peripheral blood lymphocytes, and monocytes show different migratory behaviors in EC/SMC coculture under disturbed flow. Purified neutrophils, peripheral blood lymphocytes (PBLs), and monocytes are perfused over the EC/SMC coculture in the step-flow channel, and the migration of arrested WBCs is traced for 20 min in areas *A*, *B*, *C*, and *D* (same designations as in Figs. 9–11) or under static condition after their transmigration across the EC monolayer to the underside. This figure shows the results of a representative experiment containing 8 cells migrating in area *A* and 15 cells migrating in areas *B*, *C*, and *D*, as well as under static condition. The positions of the centers of the cells are determined at 20-s intervals from 0 to 20 min, and their paths of travel are processed with a commercialized image analysis software. [From Chen et al. (76).]

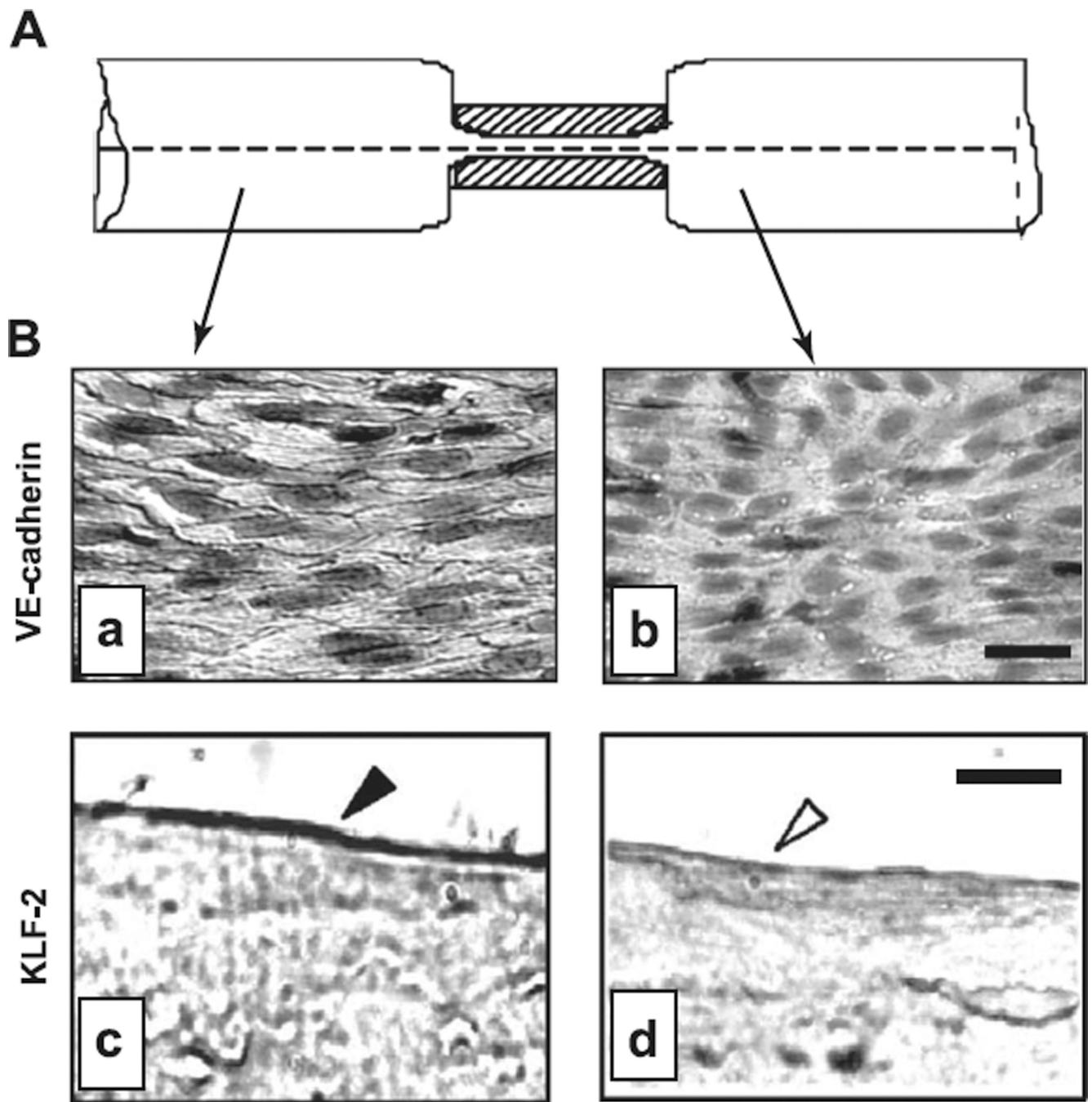


FIG. 18.

In vivo observations of VE-cadherin distribution and KLF-2 expression in response to disturbed versus laminar flows. *A*: side view of the rat abdominal aorta with a local stenosis created by using a U-shaped titanium clip, with the aim of studying the effects of flow patterns on VE-cadherin distribution and KLF-2 expression in vivo. Four weeks after the creation of stenosis, the vessel is harvested, fixed, embedded, sectioned longitudinally, and subjected to immunohistochemical staining with anti-VE-cadherin (*a* and *b*) or anti-KLF-2 (*c* and *d*) (*B*). Blood flow is from *left to right*, as indicated by the arrow. In the laminar flow region 5 mm upstream (as well as 5 mm downstream; not shown) to the clip site, VE-cadherin is highly expressed at EC borders (*B-a*), and the endothelium shows strong KLF-2

staining (closed arrowhead) (*B-c*). No detectable VE-cadherin is found at cell borders in the disturbed flow region downstream to the constriction (*B-b*). In this region, there is also a lack of KLF-2 staining (open arrowhead) (*B-d*). Bars in *B-b* and *B-d* are 30 and 10 μm , respectively. [From Miao et al. (379) and Wang et al. (600).]

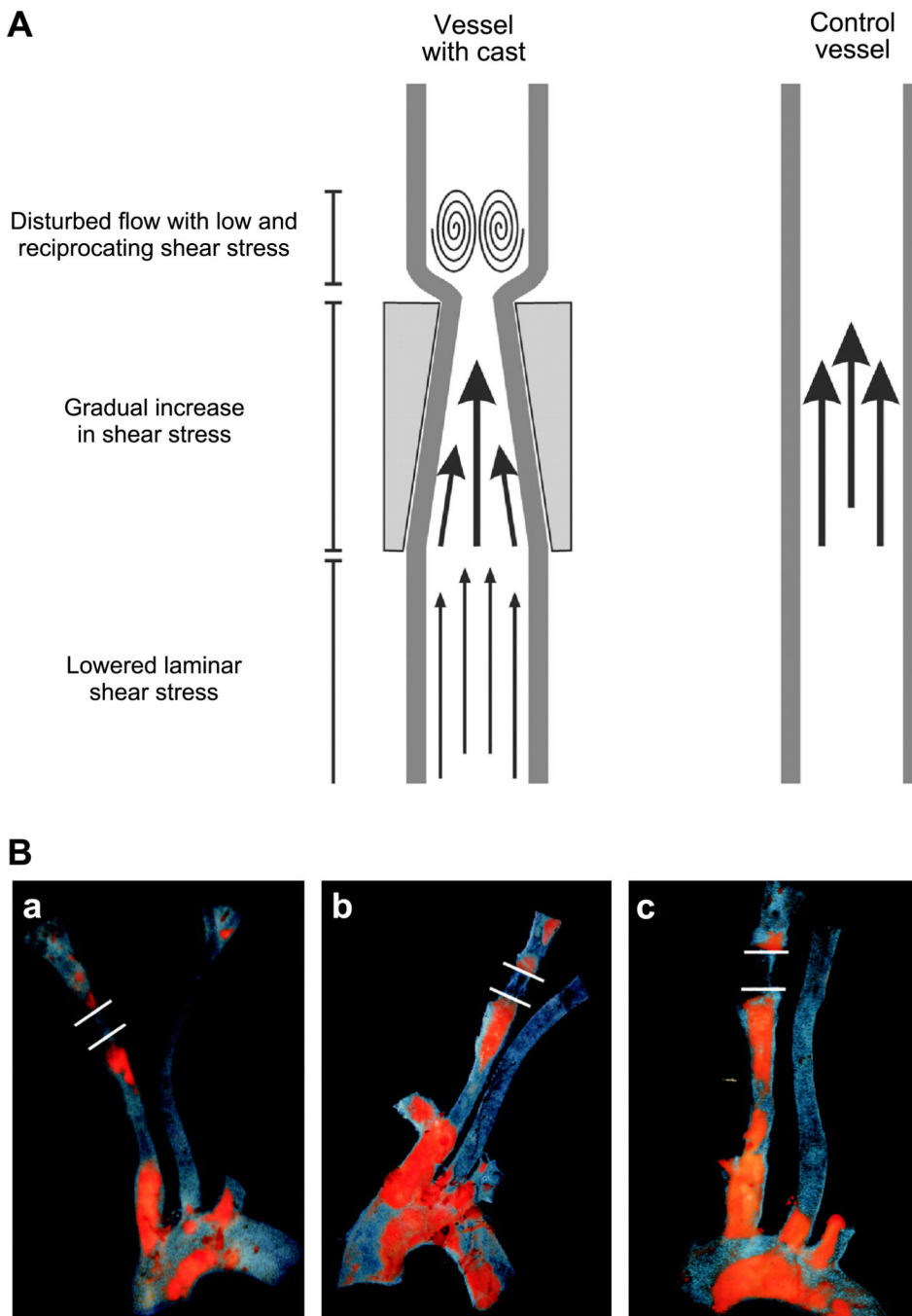


FIG. 19. Both unidirectional low shear stress and disturbed flow with low and reciprocating shear stress induce the development of atherosclerotic lesions. *A*: schematic representation of the shear stress patterns induced by the cast. On the *right* is a straight segment of a mouse common carotid artery without a cast, which has laminar blood flow (indicated by parallel arrows). On the *left* is a mouse common carotid artery with the cast. Upstream from the cast, shear stress is relatively low (compared with the shear stress in the control vessel) due to the flow-limiting stenosis induced by the cast. Within the cast, a gradual increase in shear stress occurs due to the tapered shape of the cast. A disturbed flow region with low and reciprocating shear stress occurs immediately downstream the cast. *B*: ApoE^{-/-} mice are

instrumented with the cast 2 wk after starting the atherogenic Western diet and the specimens were obtained after 6 (*B-a*), 9 (*B-b*), or 12 wk (*B-c*) of cast placement. Whole-mount aortic arches and carotid arteries are stained with Oil red O for atherosclerotic lesions. White lines demarcate the previous position of the cast with the increased shear stress region. It is noted that atherosclerosis is induced in regions of both 1) unidirectional low shear stress upstream the cast and 2) disturbed flow with low and reciprocating shear stress downstream the cast. For details, see Cheng et al. (82). [From Cheng et al. (82).]

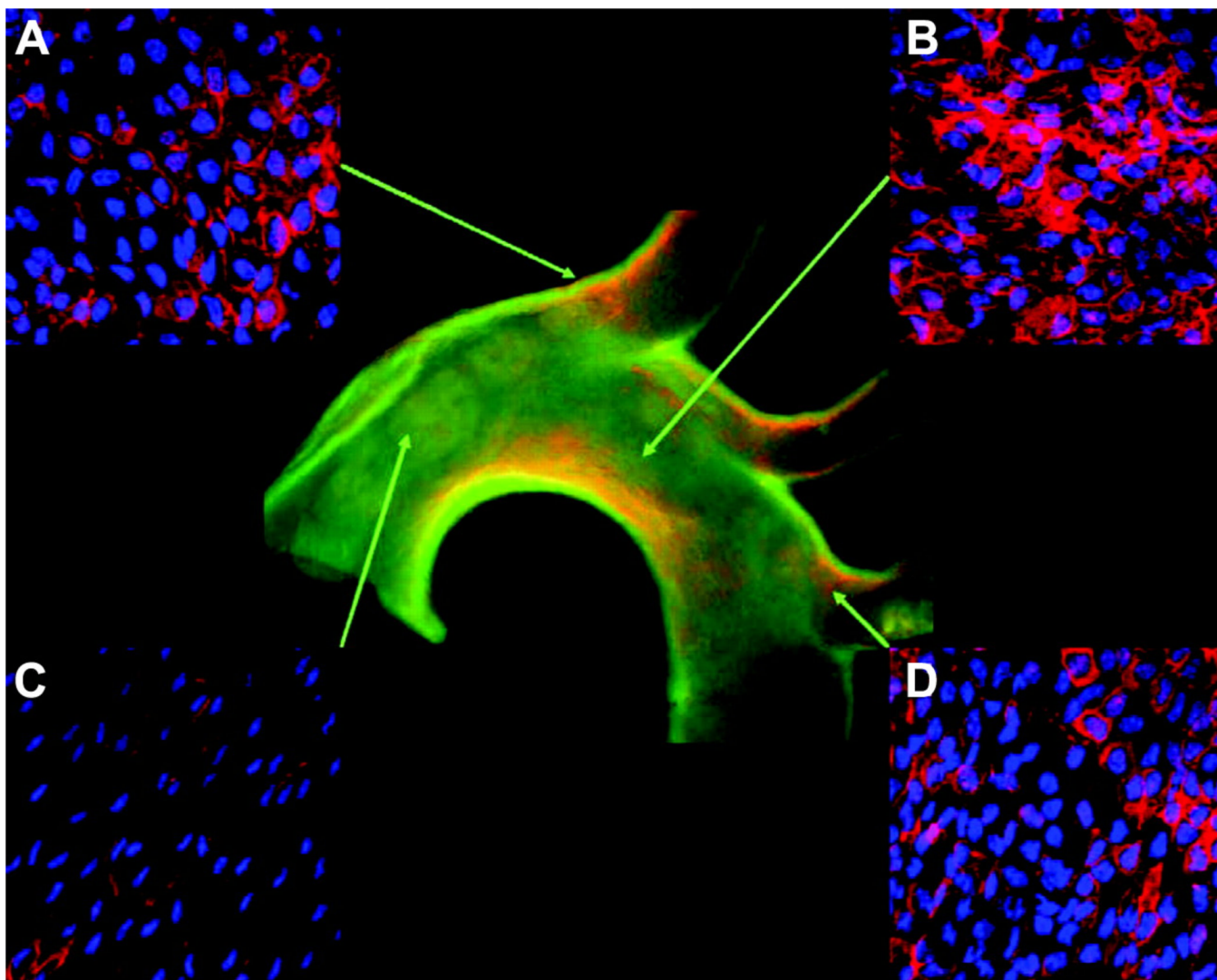


FIG. 20.

Enhanced expression of VCAM-1 along the inner curvature of the aortic arch and at the orifices of the arch branches. VCAM-1 protein expression in the mouse aortic arch is analyzed ex vivo by using immunohistochemical staining with an antibody against VCAM-1, followed by Qdot-bioconjugated secondary antibody, and two-photon excitation microscopy (center). Regions with enhanced VCAM-1 expression can be observed along the inner curvature of the aortic arch and at the orifices of the arch branches. This distribution correlates with the development of lesions (Fig. 4) and low values of mean wall shear stress (Fig. 5B). *Panels A, B, C, and D* show differential expression of VCAM-1 in different areas indicated by arrows. The lateral walls of the ascending aorta (i.e., *panel C*) are areas where there is less VCAM-1 expression, which coincides with a relatively high mean shear stress. [From Suo et al. (552).]



FIG. 21. RasN17 prevents restenosis of rat common carotid artery after balloon injury. The photographs show (*left to right*) the wide lumen in control artery, the severe stenosis following balloon injury without AdRasN17 (with only control AdLacZ), and the marked reduction of stenosis with vessel patency following balloon injury with AdRasN17 treatment. [From Chien (86).]

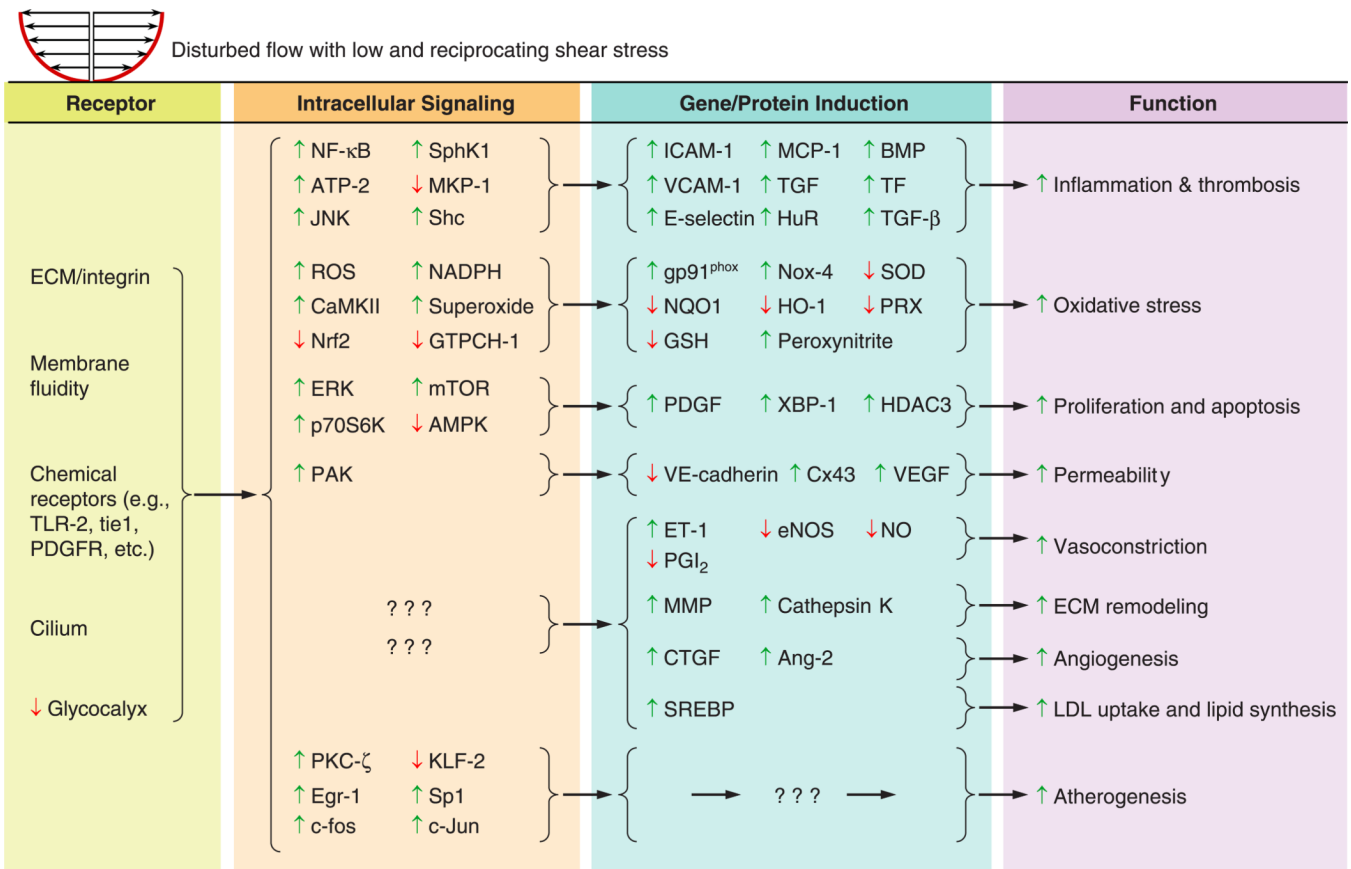


FIG. 22. EC signaling, gene expression, and function regulated by disturbed flow. ↑, Upregulation or sustention versus laminar flow with higher shear stress; ↓, downregulation versus laminar flow with higher shear stress. “???” indicates that the molecules responsible for mediating the signaling are still not known.

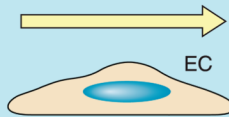
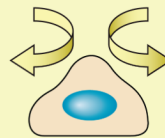
	Laminar flow / high shear stress	Disturbed flow / low or reciprocating shear stress
		
Vasoactivity	Vasodilation	Vasoconstriction
Turnover rate	Low	High
Macromolecular permeability & LDL uptake	Low	High
DNA synthesis	Low	High
Morphology	Elongated & aligned	Polygonal
Expression of adhesion molecules, inflammatory & chemokine genes	Low	High
Expression of antioxidant genes	High	Low
WBC adhesion and platelet aggregation	Inhibition	Promotion
Oxidative stress/ROS	Low	High (Sustained)
VSMC activation	Low	High
Wound repair: Endothelization	Promotion	Retardation
Heterogeneity	Low	High
Fibronectin/fibrinogen deposition	Low	High
Atherosclerosis & thrombosis	Prevention	Promotion

FIG. 23. Summary of effects of different flow patterns and associated shear stresses on endothelial and vascular biology.

TABLE 1
A selection of studies showing the correlation between locations of atherosclerotic lesions and regions of disturbed flow in human arteries

N	Subject				Methods			Reference Nos.
	Sample source	Age	Sex	Vessel	Experimental	Modeling	Flow pattern and visualization	
12	Autopsy (no history of stroke or symptoms of cerebral dis.)	27–73 (Mean: 53)	Either	Carotid artery	Angiogram, histology	Scale model, LDA	Steady (H bubble, dye injection, and washout)	644
20 (lesions)	Autopsy (no history of symptomatic cerebrovasc. dis.)	27–73 (Mean: 53)	Either	Carotid artery	Angiogram, histology	Scale model, LDA	Pulsatile, Oscillatory	311
23	Patients (with coronary heart disease risk factors and plaque in the CCA, and/or CB of one side and no lesions in the contralateral carotid tree)	Mean: 55	Either	Carotid artery (Plaque/control)	Echo-Doppler	Wall shear stress = blood viscosity × blood velocity/internal diameter		217
30	Patients (no obstruct. dis.)	34–81 (Mean: 60)	Either	Coronary artery	Angiogram, histology	Velocity assessed by the rate of clearance of contrast material		494
17	Autopsy (no card. dis.)							
5	Autopsy (no card. dis.)	M: 18, 56, 61, 75 F: 51	Either	Coronary artery	High-speed cinemicrography, histology	Isolated transparent natural arteries	Steady, pulsatile (suspended tracer particles)	16
26 (patients) 74 (lesions)	Patients (treated with diet or medication over 3 yr)		Either	Coronary artery	Angiogram	FDM	Steady	207
13	Patients (with lumen obstruction <50%)	Mean: 61	M: 9 F: 4	Coronary	Intracoronary ultrasound, angiogram	Structured grid and FVM		544
15	Autopsy	64	M	Abdominal aorta, Common iliac artery	Angiogram	Anatomically accurate model	Steady, pulsatile (dye injection and washout)	595
10	Autopsy (no clinical symptoms referable to the aorta)	41–92 (Mean: 55)	M	Abdominal aorta	Angiogram, histology, quantitative morphometry	Anatomically accurate model, MRI velocimetry	Pulsatile, oscillatory	392
10	Autopsy (no history of cardiovasc. dis.)	18–40 (Mean: 34)	M: 9 F: 1	Abdominal aorta	Histology	Anatomically accurate model, LDA	Pulsatile	454
10	Autopsy (no history of cardiovasc. dis.)	18–40 (Mean: 34)	Either	Abdominal aorta	Histology	MRI velocimetry	Pulsatile	455
26	Patients (hyperlipidemic; lipid-lowering therapy; all had either intermittent claudication or asymptomatic hypercholesterolemia combined with pathological findings at plethysmography and slight or moderate femoral atherosclerosis)	39–69	Either	Femoral artery	Cineangiography	Linear-regression analysis	Pulsatile (contrast injection, ZDF)	535
17	Patients (hyperlipidemic; lipid-lowering therapy; all had either intermittent claudication or asymptomatic hypercholesterolemia combined with pathological findings at plethysmography and slight or moderate femoral atherosclerosis)	39–69	M: 7 F: 10	Femoral artery	Cineangiography		Pulsatile (contrast injection, ZDF)	534

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

CB, carotid bulb; CCA, common carotid artery; F, female; FDM, finite difference method; FVM, finite volume method; LDA, laser-Doppler anemometry; M, male; MRI, magnetic resonance image; ZDF, zones of delayed contrast filling.

TABLE 2

Summary of studies using step-flow chamber on endothelial responses to laminar and disturbed flows

Responses	Laminar Flow (Shear Stress >10 dyn/cm ²)	Disturbed Flow (Shear Stress ~ ± 4 dyn/cm ²)	Reference Nos.
Morphology	Aligned	Round	94, 147
Actin distribution	Long and parallel stress fibers in central regions	Random and short filaments at cell periphery	94
Proliferation/DNA synthesis/mitosis	Low	High	94, 86, 148, 337, 564
Migration	Fast	Slow	259
Permeability	Low	High	458
Signaling; gene expression, and production			
Gene expression heterogeneity	Low	High	134
Connexin43	Low, continuous distribution	High, discontinuous distribution	134, 147
Nuclear factor- B	Low	High	405
Early growth factor-1	Low	High	405
<i>c-fos</i>	Low	High	405
c-Jun	Low	High	405
Intercellular adhesion molecule-1	Low	High	88
E-selectin	Low	High	88
<i>Tie1</i>	Low	High	469
VE-cadherin	Continuous distribution	Discontinuous distribution	379
Sterol regulatory element binding protein	Transient activation	Sustained activation	349
WBC adhesion	Low	High	28, 88, 76, 532
WBC transendothelial migration	Low	High	76

WBC, white blood cell.

TABLE 3

In vivo correlations of in vitro responses of EC signaling and gene expression to disturbed flow at athero-prone and poststenotic areas

Signaling/Genes and Products (Expression or Activity)	In vitro				In vivo				Refs.
	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	Model	Stimulus		Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject		
			Flow type: dyn/cm ²	Duration (h)				Reference Nos.	
Inflammation									
ICAM-1	Increased	HUVEC in parallel-plate chamber	PF: 5 ± 5	24	72	Profound focal expression in the outer wall of the internal carotid artery and advanced lesions	Carotid artery	Patients	168
			RF: ± 5						
	Increased	EC in orbital shaker/parallel-plate chamber	LF: 14	6	119	Highly expressed at lesion-prone sites	Aortas	C57BL/6 mice	269
			Orbital: 4–12 (96–210 rpm)						
	Increased	BAEC in flow channel	PF: 50 ± 40	4	257	Highly expressed at lesion-prone sites	Ascending aorta and proximal arch	ApoE ^{-/-} mice	407
			RF: ± 2.6						
	Increased	HAEC in cone-and-plate viscometer	RF: ± 5	24	536	Increased by disturbed flow induced by partial ligation	Carotid artery	C57BL/6 mice	410
VCAM-1	Increased	HAEC in cone-and-plate viscometer	LF: 5, 15	24	537	Highly expressed at lesions	Coronary artery	Patients	536
			RF: ± 5						
	Increased	HAEC in disturbed flow/parallel-plate chamber	LF: 13	24	50	Expressed at advanced lesions	Carotid artery	Patients	168
			Recirculation: < 0.01						
	Increased	HUVEC in parallel-plate chamber	PF: 5 ± 5	24	72	Highly expressed at lesion-prone sites	Aortas, Ascending aorta and proximal arch, Aortic arch	C57BL/6 mice	269
			RF: ± 5						
	Increased	HUVEC in cone-and-plate viscometer	Athero-prone versus atheroprotective patterns form human carotid	24	115	Highly expressed at lesion-prone sites	Aortic sinus, ascending aorta, aortic arch, and abdominal aorta	ApoE ^{-/-} mice	407

Signaling/Genes and Products (Expression or Activity)	In vitro				In vivo				Refs.
	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	Model	Stimulus Flow type: dyn/cm ²	Duration (h)	Reference Nos.	Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject	
Increased	HUVEC in cone-and-plate viscometer		24	239		Increased by disturbed flow induced by partial ligation	Carotid artery	C57BL/6 mice	410
Highly expressed in lesser curvature and orifices of arch branches	C57BL/6 mice	552						ApoE ^{-/-} mice	
Increased expression with reduced flow by ligation	Rabbits	596						p47 ^{phox} ^{-/-} mice	
E-selectin	HAEC in disturbed flow/parallel-plate chamber	LF: 13 Recirculation: <0.01	24	52		Expressed at advanced lesions	Carotid artery	Patients	168
Increased	HUVEC in parallel-plate chamber	PF: 5 ± 5 RF: ± 5	24	72					
Increased	EC in orbital shaker/parallel-plate chamber	LF: 14 Orbital: 4–12 (960–210 rpm)	6	119					
MCP-1	BAEC in flow channel	PF: 50 ± 40 RF: ± 2.6	4	257		Highly expressed near the intercostal artery orifices	Descending aorta	Rats	86
Increased	BAEC in flow channel	LF: 25 RF: ± 3	4, 8	265					
BMPs or their antagonists	BAEC/HCAEC in cone-and-plate viscometer	LF: 15 RF: ± 5	24	71		Highly expressed at lesions	Carotid artery	Patients	46
Increased	MAEC in cone-and-plate viscometer	LF: 5, 15 RF: ± 5	24	537		Coexpression of BMP-4 and the antagonists follistatin, noggin, and matrix Gla protein	Aortic arch and thoracic aorta Coronary artery	C57BL/6 mice	71
						Sustained immunoreactivity of matrix Gla protein at lesions	Abdominal aorta	Patients	Patients
						Increased for BMP-4 by disturbed flow induced by partial ligation	Carotid artery	C57BL/6 mice ApoE ^{-/-} mice	151 410

Signaling/Genes and Products (Expression or Activity)	In vitro				In vivo				Refs.
	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	Model	Stimulus		Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject		
			Flow type: dyn/cm ²	Duration (h)				Reference Nos.	
TLR-2	Increased	HCAEC in step flow chamber	LF: >5	12	161	Highly expressed at lesions	Coronary artery	p47 ^{phox} ^{-/-} mice	537
JNK	Increased	BAEC in parallel-plate chamber	Recirculation LF: 12	18	229	Highly expressed in lesser curvature Atheroprotective by TLR2 deficiency Higher phosphor. in lesser curvature versus greater curvature of aortic arch	Aortic arch Aortic arch Aortic arch	LDLR ^{-/-} mice TLR2 ^{-/-} mice C57BL/6 mice	399 229
HuR	Increased	HUVEC in cone-and-plate	RF: 0.6 ± 1.92			Higher phosphor. in carotid sinus versus straight segments	Carotid artery	ApoE ^{-/-} mice	487
Oxidative stress Superoxide, NAD(P)H oxidase, H ₂ O ₂ , orperoxynitrite	Increased	HUVEC in parallel-plate chamber	LF: 5 RF: ± 5	1, 5, 24	138	Increased in lesser curvature versus greater curvature of aortic arch, and by disturbed flow induced by partial carotid ligation	Aorta arch Carotid artery	C57BL/6 mice	487
	Increased	BAEC in a flow channel	LF: 23	4	258				
	Increased	BAEC in flow channel	RF: 0.02 ± 3	4, 8	266				
	Increased (Xanthine oxidase)	BAEC/MAEC in cone-and-plate viscometer	LF: 15 RF: ±15	4	374				
GTPCH-1	Decreased	HAEC/HUVEC in cone-and-plate viscometer	LF: 15 RF: ± 15	14	335	Decreased by disturbed flow induced by partial ligation	Carotid artery	C57BL/6 mice	335
Regulation of endothelial integrity/apoptosis									
XBP-1	Increased	HUVEC in parallel-plate chamber	LF: 12 RF: ± 4.5	24	647	Highly expressed in branch curve and lesion areas	Aortas	ApoE ^{-/-} mice	647
Tie1	Increased	BAEC in a parallel-plate/step flow chamber	LF:12	16	469	Up-regulated at the (1) femoral artery and its branches, (2) aorta-renal artery junction, (3) inner aspect of the	Femoral artery, aorta-renal artery junction,	<i>tie</i> ⁻ / <i>lacZ</i> transgenic rats,	469

Signaling/Genes and Products (Expression or Activity)	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	In vitro				In vivo				Refs.
		Model	Stimulus		Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject	Refs.		
			Flow type: dyn/cm ²	Duration (h)					Reference Nos.	
HDAC3	Increased	HUVEC in orbital shaker	Recirculation	0–20 with mean of 4.5 at 0.5Hz	24	643	cup-shaped cusps in the aortic side of aortic valve, (4) atherosclerotic lesions in the abdominal aorta, (5) downstream of the A/V junction in vein interposition grafts, and (6) region of flow obstruction in aneurysms.	aortic valve, abdominal aorta, vein interposition grafts, aneurysms	<i>tie1-lacZ</i> transgenic mice, ApoE ^{-/-} mice	643
Regulation of endothelial permeability										
PAK	Increased	BAEC in parallel-plate flow chamber/cone-and-plate viscometer	LF: 12 Athero-prone versus atheroprotective patterns from human carotid	4	4	440	PAK activity correlates with VCAM-1 expression, FN deposits, and vascular permeability	Carotid artery, Aortic arch	ApoE ^{-/-} mice	440
Cx43	Increased	BAEC in step flow channel	LF: 13.5 Recirculation: 0–8.5	5, 16	147	147	Abundant at downstream edge of the ostia of branching vessels and at flow dividers	Aortas	Rats	195
Regulation of EC migration										
Ang-2	Increased	HUVEC/HMEC/BAEC in cone-and-plate viscometer	LF: 5, 15 RF: ± 5, ± 15	24	574	574	Highly expressed at lesions	Aortic arch Carotid artery	LDLR ^{-/-} mice Patients	314
Regulation of SMC proliferation and migration										
PDGF-A, -B or receptors	Increased by TF versus LF and PF, Decreased by LF versus static	BAEC in cone-and-plate viscometer	LF: 15, 36 TF: 15 ± 0.90 PF: 12 ± 0.06–18 ± 0.29	6	361	361	Increased in lesser curvature versus greater curvature of aortic arch	Carotid artery	Patients	616
PDGF-DD	Increased	HUVEC/HUVSMC in cone-and-plate viscometer	Athero-prone versus atheroprotective patterns from human carotid	24	566	566	Increased at lesions	Aortic arch	ApoE ^{-/-} mice	566
Regulation of ECM										

Signaling/Genes and Products (Expression or Activity)	In vitro				In vivo				Refs.
	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	Model	Stimulus		Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject	Reference Nos.	
			Flow type: dyn/cm ²	Duration (h)					
Cathepsin K	Increased	MAEC in cone-and-plate viscometer	LF: 15 RF: ± 5	24	Positive correlation with elastic lamina fragmentation	Coronary arteries with various degrees of plaques	Patients	461	461
MMP-9	Increased	Perfused left common porcine carotids <i>ex vivo</i>	PF: 0.3 ± 0.1, 6 ± 3 RF: 0.3 ± 3	72	Increased by flow cessation by ligation	Carotid artery	C57BL/6J mice	200	218
MMP-2	Increased	MLEC/HUVEC in cone-and-plate viscometer	LF: 15 RF: ± 15	6	Increased by reduction in flow by partial outflow occlusion in injured right common carotid artery	Carotid artery	New Zealand White male rabbits	200	29
Vasodilator	Decreased	Perfused left common porcine carotids <i>ex vivo</i>	PF: 0.3 ± 0.1, 6 ± 3 RF: 0.3 ± 3	72	Low expression at poststenotic sites	Carotid artery constriction	eNOS-GFP transgenic mice	199	83
eNOS	Decreased	BAEC in flow channel	LF: 25 RF: ± 3	4, 8	Decreased by disturbed flow induced by partial ligation	Carotid artery	C57BL/6 mice ApoE ^{-/-} mice p47 ^{phox} ^{-/-} mice p47 ^{phox} ^{-/-} /ApoE ^{-/-} mice	258	410
	Decreased	BAEC in flow channel	LF: 10 RF: 10 ± 10	4				474	
	Decreased	Perfused BAEC tubes	PF: 6 ± 3 RF: 0.3 ± 3	4, 24				526	
	Decreased	Perfused EAhy 926 tubes	PF: 6 ± 3 RF: 0.3 ± 3	24				527	
	Decreased	HAEC in cone-and-plate viscometer	LF: 15 RF: ± 15	14				615	
Complement inhibitory protein	Decreased	Perfused BAEC tubes	PF: 6 ± 3 RF: 0.3 ± 3	1, 4, 24				653	

Signaling/Genes and Products (Expression or Activity)	In vitro				In vivo				Refs.
	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	Model	Stimulus		Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject		
			Flow type: dyn/cm ²	Duration (h)				Reference Nos.	
CD59	Decreased versus LF and no induction versus static	HUVEC/HAEC in parallel-plate chamber	LF: 12 RF: ±5	24, 48	296	Aortas	Murine	296	
Signal transduction									
AMPK	Decreased	BAEC in parallel-plate chamber	LF: 12 RF: 1 ± 5	8	228	Aorta arch Thoracic aorta	C57BL/6J mice	228	
p70S6K	Increased	BAEC in parallel-plate chamber	LF: 12 RF: 1 ± 5	8	228	Aorta arch Thoracic aorta	C57BL/6J mice	228	
MKP-1	Increased by LF versus static	HUVEC in parallel-plate chamber	LF: 12	24, 48	642	Aortas	C57BL/6 mice MKP-1 ^{-/-} mice	642	
Shc	Increased	BAEC in parallel-plate chamber	LF: 12 RF: ±6.5	18	350	Aortas	C57BL/6 mice	350	
Transcription factor									
KLF-2	Decreased	HUVEC in cone-and-plate viscometer	Athero-prone versus atheroprotective patterns from human carotid	24	444	Abdominal aorta bifurcation, common iliac artery, carotid artery, bifurcation, aortic arch, carotid artery constriction	Patients ApoE ^{-/-} mice	145	
	Decreased	HUVEC in parallel-plate chamber	PF: 12 ± 4 RF: 0.5 ± 4	24	600	Carotid artery	C57BL/6 mice ApoE ^{-/-} mice p47 ^{phox} ^{-/-} mice p47 ^{phox} ^{-/-} /ApoE ^{-/-} mice	410	
Nrf2	Decreased	HUVEC in cone-and-plate viscometer	Athero-prone versus atheroprotective patterns from human carotid	24	116	Abdominal aorta and celiac artery, Abdominal aorta constriction	Rats	600	
NF- B	Increased	HUVEC in cone-and-plate viscometer	Athero-prone versus atheroprotective patterns from human carotid	24	115	Atherosclerotic aortas, intima/media and atheromatous areas of lesion	Patients	48	

Signaling/Genes and Products (Expression or Activity)	In vitro				In vivo				Refs.
	Effect of Reciprocating or Low Flow (Versus Laminar or Pulsatile)	Model	Stimulus		Response (Versus Straight Segment or Control Vessel)	Segment	Animal/Subject		
			Flow type: dyn/cm ²	Duration (h)				Reference Nos.	
	Increased	HUVEC in cone-and-plate viscometer	Athero-prone versus atheroprotective patterns from human circulation	24	239	Highly expressed at lesion-prone sites	Ascending aorta and arch	C57BL/6 mice	234
	Increased by 2 versus 16 (dyn/cm ²)	HAEC in parallel-plate chamber	LF: 2, 16	0.5–24	387	Robust p65 localization in lesser curvature versus greater curvature	Aortic arch	ApoE ^{-/-} mice	239
	Increased	BAEC/HUVEC in parallel-plate chamber	LF: 12	18	437	Activated (inhibited by PAK-Nck blocking peptides)	Carotid sinuses	C57BL/6 mice	437
Egr-1	Increased	HUVEC in step flow chamber	RF: 1 ± 12		405	Activated in experimental hypercholesterolemia	Coronary arteries	Pigs	629
Others						Highly expressed at lesions	Carotid artery, Aortas	Patients	369
Heat shock protein 60	Decreased by low shear versus high shear.	HUVEC in cone-and-plate viscometer	LF: 0–50	24	252	Lower expression in reduced shear versus increased shear regions by ligation	Carotid artery	Lewis rats	252

BAEC, bovine aortic endothelial cell; HAEC, human aortic endothelial cell; HCAEC, human coronary artery endothelial cell; HMEC, human microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; HUVSMC, human umbilical vein smooth muscle cell; MAEC, mouse aortic endothelial cell; MLEC, murine lymphoid endothelial cell; LF, laminar flow; RF, reciprocating flow; PF, pulsatile flow; TF, turbulent flow; FN, fibronectin.