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Blood flow and arterial endothelial dysfunction: Mechanisms and implications

Écoulement sanguin et dysfonctionnement de l'endothélium artériel : mécanismes et implications

Abdul I. Barakat

Hydrodynamics Laboratory (LadHyX), CNRS UMR 7646, École polytechnique, route de Saclay, 91128 Palaiseau cedex, France

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ABSTRACT

The arterial endothelium exquisitely regulates vascular function, and endothelial dysfunction plays a critical role in the development of atherosclerosis. Atherosclerotic lesions develop preferentially at arterial branches and bifurcations where the blood flow is disturbed. Understanding the basis for this observation requires elucidating the effects of blood flow on the endothelial cell (EC) function. The goal of this review is: (1) to describe our current understanding of the relationships between arterial blood flow and atherosclerosis, (2) to present the wide array of flow-induced biological responses in ECs, and (3) to discuss the mechanisms by which ECs sense, transmit, and transduce flow-derived mechanical forces. We conclude by presenting some future perspectives in the highly interdisciplinary field of EC mechanotransduction.

RÉSUMÉ

L'endothélium artériel régule finement la fonction vasculaire, et le dysfonctionnement de l'endothélium joue un rôle essentiel dans le développement de l'athérosclérose. Les lésions d'athérosclérose se développent préférentiellement au niveau des branches et des bifurcations artérielles, là où le flux sanguin est perturbé. Comprendre la base de cette observation nécessite d'élucider les effets de l'écoulement sanguin sur la fonction des cellules endothéliales (CE). Le but de cette revue est : (1) de décrire notre compréhension actuelle de la relation entre l'écoulement sanguin artériel et l'athérosclérose, (2) de présenter le large éventail des réponses biologiques des CE induites par l'écoulement, et (3) de discuter les mécanismes par lesquels les CE sentent, transmettent, et traduisent les forces mécaniques générées par l'écoulement. Nous conclurons en présentant quelques perspectives dans le domaine hautement interdisciplinaire de la mécanotransduction des CE.

E-mail address: Abdul.Barakat@ladhyx.polytechnique.fr.

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1. Introduction and scope

The endothelium is the cellular monolayer that lines the inner surfaces of all blood vessels. In the arterial system, endothelial cells (ECs) play a critical role in regulating normal vascular function, and endothelial dysfunction is centrally implicated in the development and progression of atherosclerosis and diabetes. In vivo, vascular ECs encounter a multitude of biochemical stimuli as a result of their interactions with small and large molecules in the bloodstream as well as with soluble factors released by blood cells and by other cells of the arterial wall, most notably smooth muscle cells. These stimuli trigger intracellular signaling pathways that regulate vascular structure and function. In addition to biochemical stimuli, however, arterial ECs are constantly subjected to a broad array of biophysical stresses on both their apical and basal surfaces. On their apical side, ECs are subject to pressure forces and to fluid dynamic shear stresses due to the flow of viscous blood. On their basal side, ECs sense and respond to the roughness and rigidity of their substrate [1,2]. Both the apical and basal EC surfaces also experience significant stretch forces due to the pressure difference across the compliant arterial wall.

Research over the past two decades has demonstrated that biophysical forces, most notably flow-derived forces, exquisitely regulate vascular structure and function [3–6]. The current view is that the endothelium is a natural mechanotransducer, a structure that senses mechanical forces on its surface and transduces these forces into biochemical signals. There is a large body of literature on the wide variety of biological responses that flow induces in ECs. These studies have demonstrated that flow can trigger many of the same signaling cascades that are activated by soluble cytoactive factors, with resulting activation of transcription factors that ultimately regulate gene expression and protein synthesis. In addition to occurring through signaling pathways that are common to biochemical signaling, however, mechanical signal transduction in ECs can also occur through seemingly unique pathways. Biochemical signaling cascades consist of series of consecutive reaction–diffusion processes that are relatively slow, often requiring tens of seconds to propagate through a distance comparable to the length of a cell. Mechanical signals, on the other hand, can be transmitted over similar distances much more rapidly (within ∼100 ms) [7]. It has been proposed that the complex network of cytoskeletal filaments within ECs provides direct physical connections that allow very rapid long-distance transmission of mechanical signals [3,8–11]; however, how exactly this transmission occurs remains poorly understood. The discrete nature of cytoskeletal architecture may also facilitate spatial focusing of the imposed mechanical signal and its delivery to specific target sites within the intracellular space, thereby limiting mechanical energy dissipation within the cytosol and hence maximizing “bang for the buck”.

Despite our seemingly ever-expanding knowledge of the variety of EC biological responses to flow, much remains unknown about how ECs sense flow, how they transmit the flow signal to a target intracellular site, and how the signal is ultimately transduced into a biochemical message. Thus, there is great current interest in the mechanisms that govern mechanosensing, mechanotransmission, and mechanotransduction in vascular ECs. Experiments have also demonstrated that beyond being simply responsive to flow, ECs respond differently to different flow waveforms. For instance, while steady flow induces EC elongation, cytoskeletal remodeling, and mobilization of intracellular calcium, purely oscillatory flow (periodic oscillations about a zero mean) fails to elicit any of these responses [12–15]. Steady flow and oscillatory flow also have different effects on flow-activated ion channels [16,17]. Therefore, understanding EC responsiveness to flow also requires elucidating the mechanisms governing mechano-differentiation, i.e. how ECs distinguish among different flow waveforms. As will be discussed in more detail later, understanding the basis of EC mechano-differentiation is critical for understanding the involvement of flow in the development and progressions of vascular pathology.

In this review, we begin by outlining some relevant physical considerations about the vascular endothelium and by briefly describing the importance of flow in regulating EC function and dysfunction. We then summarize the current state of knowledge of EC biological responses to flow and their implications for vascular disease, most notably atherosclerosis. We subsequently embark on a description of mechanisms that govern mechanosensing with a presentation of candidate mechanosensors and how some of these sensors may endow ECs with the ability to discriminate among different flow waveforms. This is followed by a discussion of mechanotransmission with particular focus on the notion of rapid, long-distance, and targeted force transmission via the cytoskeleton. We then provide some general thoughts on possible mechanisms by which mechanical stimuli are converted to biochemical signals and conclude with some future perspectives in this highly dynamic and interdisciplinary field of study.

2. Arterial endothelium: structural and physical considerations

We begin by discussing some physical considerations. Much of what we know about the physical attributes of vascular ECs comes from in vitro studies involving cells in culture. In static culture, arterial ECs form continuous monolayers within which individual cells take on a characteristic cobblestone morphology with an average cellular diameter of ∼25–30 μm (Fig. 1A). The cytoskeletal organization of these cuboidal cells is typically characterized by a dense band of cortical actin as well as prominent, straight, and largely parallel actin stress fibers that are generally oriented at different angles in the different cells within the monolayer (Fig. 1B). Because ECs in vivo are constantly under flow, many studies have investigated the effect of flow on EC morphology and cytoskeletal organization. These studies have demonstrated that under sufficiently high levels of fluid dynamic shear stress, ECs become highly elongated (cell length ∼50–60 μm) and aligned in the flow direction (Fig. 1A). Flow-elongated ECs appear to lack the dense cortical actin band, and they exhibit stress fibers that are aligned in

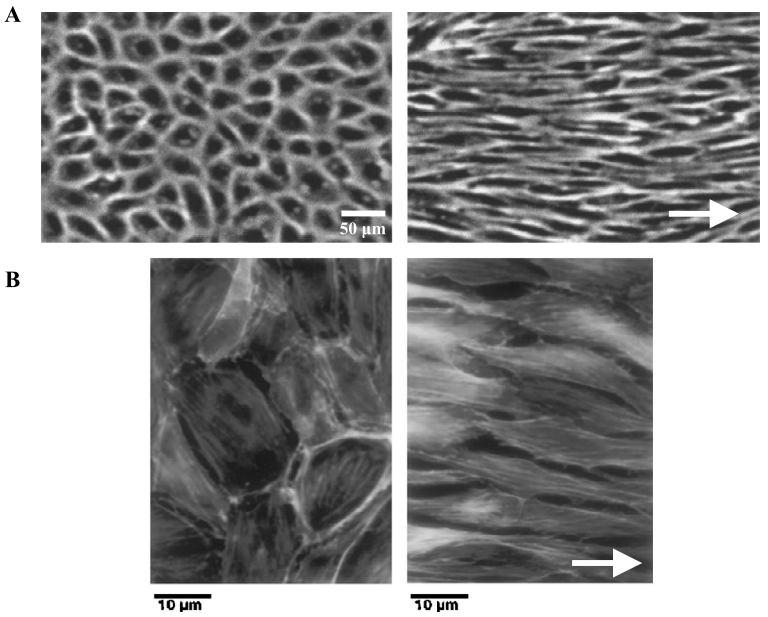

Fig. 1. Effect of steady flow on EC morphology and filamentous actin (F-actin) organization. A: Bovine aortic ECs before flow (left panel) and after the application of a steady shear stress of 19 dyne/cm^2 for 24 h in the direction of the white arrow (right panel). The cells elongate and align in the direction of flow. Taken from [15]. B: F-actin organization in bovine aortic ECs before flow (left panel) and after the application of a steady shear stress of 13 dyne/cm^2 for 24 h in the direction of the white arrow (right panel). Note extensive F-actin remodeling. Taken from [18].

the flow direction (Fig. 1B). The potential implications of differences in cytoskeletal architecture for EC mechanotransduction will be discussed in a later section.

ECs, of course, are three-dimensional; however, they are very flat. More specifically, ECs in vitro are only ∼2–4 μm high at their tallest point above the nucleus (and much thinner towards the cell periphery) [19]. ECs are anchorage-dependent cells that can only survive and function normally when firmly adherent to a substrate. In medium and large arteries, the endothelial substrate is a basement membrane that consists of a complex mixture of various extracellular matrix proteins including collagen, laminin, fibronectin, and vitronectin along with glycoproteins (such as entactin) and heparan sulfate proteoglycans, most notably perlacan [20]. It is important to recognize that the endothelial basement membrane is far from a smooth surface. Rather, it is a patterned rough surface with nano-scale topographical features [21]. As in other cell types, ECs attach to the extracellular matrix via discrete focal adhesion sites on the basal cell surface. These sites, which are highly dynamic, consist of transmembrane integrin proteins that connect to the intracellular cytoskeleton via linker proteins such as vinculin, talin, and α-actinin [22]. ECs are also contact-inhibited cells; thus, they proliferate rapidly when in a sub-confluent state, but their proliferation rates decrease dramatically as they make contact with adjacent cells. When ECs are in contact, they form well developed cell–cell junctions that play a critical role in regulating transport across the endothelial monolayer as will be discussed in more detail in a later section.

Similar to most biological tissues, ECs exhibit viscoelastic mechanical properties. This behavior appears to be primarily driven by the complex network of cytoskeletal filaments crisscrossing ECs as well as by the cell nucleus [23–25]. Similarly to many other cell types, the cytoskeleton in ECs consists of three primary filament networks: actin filaments that often bundle together to form thick stress fibers (these stress fibers become even more prominent when the cells are subjected to flow), microtubules, and intermediate filaments. Fig. 2 provides an example of the very complex organization of these three filament networks in bovine aortic ECs that are cultured in the laboratory. A variety of linker proteins provide possible physical connections among the different filament networks. For instance, a number of proteins that associate with both actin filaments and microtubules have been suggested to link the two filament networks [26]. The physical links among the different cytoskeletal filament networks in ECs remain poorly characterized and certainly merit further investigation. It is recognized, of course, that the dynamic nature of the cytoskeleton and the abundant expression of cytoskeletal filaments render such studies extremely challenging.

From a mechanical point of view, actin filaments, microtubules, and intermediate filaments have largely similar effective Young's moduli of ∼1–2 GPa [27]. On the other hand, the three filament types differ significantly in their bending stiffness with values of $\sim 7 \times 10^{-26}$, $\sim 3 \times 10^{-23}$, and $\sim 4 \times 10^{-27}$ N m^2 for actin filaments, microtubules, and intermediate filaments, respectively [27]. Equivalently, the persistence lengths of actin filaments, microtubules, and intermediate filaments are respectively ∼15, ∼6000, and ∼1 μm [27], reflective of the fact that microtubules are by far the most rigid cytoskeletal element. This is thought to be attributable at least in part to the larger diameter of microtubules (∼25 nm vs. ∼6–10 nm for the other filaments). As already mentioned, actin filaments often bundle into stress fibers; these stress fibers are large

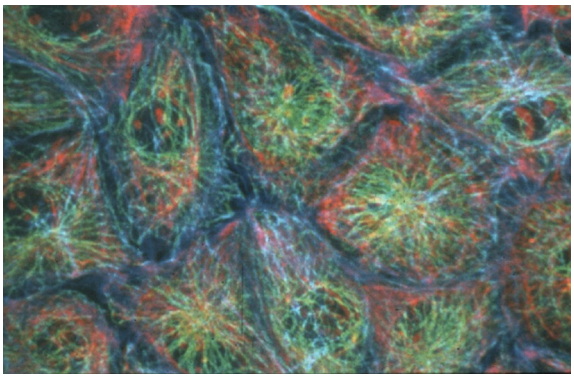

Fig. 2. Organization of the three primary cytoskeletal filament networks in bovine aortic ECs. The cells are triple stained for actin (blue); microtubules (green), and intermediate filaments (red). Taken from [5].

and very rigid structures with fiber diameter of $\sim$200 nm, bending stiffness of $\sim 10^{-22}$ N $\cdot$ m^2, and a persistence length of $\sim$20 mm [28–31].

The endothelium is not the only cell type present in the arterial wall. The medial and adventitial layers of the arterial wall contain smooth muscle cells and fibroblasts. There is evidence that both of these cell types are also mechano-responsive [32]. Because they are embedded deeper within the arterial wall, smooth muscle cells and fibroblasts are not directly exposed to blood flow. However, it has been suggested that smooth muscle cells in the media experience sufficiently elevated shear stresses due to interstitial flow to elicit significant biological signaling [32,33]. The same would presumably be true for fibroblasts in the adventitia. In any case, the different cell types within the arterial wall are in constant communication via the convective and diffusive transport of soluble factors released by the different cells. Therefore, a complete and integrated understanding of arterial EC responsiveness to flow requires establishing the effect of flow on the release of soluble factors from the different cell types in the arterial wall as well as accounting for the effects of communication among the various cell types present in the wall.

3. Role of arterial endothelium in normal vascular function

In the arterial system, the endothelium plays a number of roles that are essential for normal vascular function. A key role for endothelium is that it provides a non-thrombogenic surface (i.e. a surface that prevents blood coagulation or clotting). This is accomplished via release by ECs of nitric oxide and prostacyclin, two potent inhibitors of platelet activation and aggregation [34,35]. Endothelial damage and/or dysfunction compromises vascular non-thrombogenicity. A prominent example is following deployment of endovascular stents that massively denude the endothelium and are thus associated with a significantly increased risk of vascular thrombosis.

Another key role for the endothelium is the regulation of vascular permeability to both small solutes and macromolecules as well as to leukocyte transmigration. In a normal endothelium, intercellular junctions provide a tight permeability barrier that excludes molecules with a Stokes–Einstein radius larger than $\sim$1 nm. Therefore, large blood-borne macromolecules such as lipoproteins are generally unable to cross intercellular junctions. This is particularly significant from the standpoint of the development of atherosclerosis because lipoproteins are the primary carrier of cholesterol in the bloodstream. The molecular structure of endothelial cell–cell junctions has been and continues to be extensively studied. Although a detailed discussion of this topic is beyond the scope of the present review, we simply note that there are three types of intercellular junctions present in arterial ECs: adherens junctions, tight junctions, and gap junctions. Each of these junctions has its own distinct molecular structure. Adherens junctions consist primarily of transmembrane vascular endothelial (VE)-cadherin proteins and associated intracellular partners including platelet endothelial cell adhesion molecular-1 (PECAM-1), catenin, and plakoglobin [36]. Tight junctions consist of several families of transmembrane proteins including the occludins, claudins, and Junctional Adhesion Molecules (JAMs) as well as a number of associated intracellular partners, most notably the Tight Junction Proteins ZO-1, -2, and -3 [36]. Finally, gap junctions in ECs are comprised of three connexin (Cx) proteins (Cx37, Cx40, and Cx43) which are organized in channel-like intercellular structures called connexons [36].

In addition to their distinct structures, the different types of intercellular junctions in ECs possess distinct and specialized functions. In the case of adherens junctions, VE-cadherin links to the intracellular actin filament network; therefore, these junctions play an important role in structural stabilization and anchoring of ECs and possibly in the propagation and distribution of mechanical signals. Tight junctions are the intercellular structures that regulate solute and macromolecular transport and are therefore the primary determinant of endothelial barrier function. In the case of gap junctions, the connexons form specialized passages that permit cell–cell communication via the exchange of ions and small molecules. Inflammation compromises the endothelium's ability to act as an effective permeability barrier; therefore, vascular permeability to both small and large molecules (and possibly to leukocyte transmigration) is greatly enhanced in pathologies that involve inflammation of the endothelium such as atherosclerosis.

In addition to preventing thrombosis and regulating vascular permeability, the arterial endothelium also modulates vascular responses to changes in blood flow. In vivo, an acute increase in arterial blood flow leads to a rapid increase in arterial diameter (vasorelaxation), whereas an acute reduction in blood flow has the opposite effect (vasoconstriction) [37]. Chronic changes in arterial blood flow lead to profound structural remodeling of the arterial wall [38]. Importantly, both flow-mediated vasoregulation and flow-induced arterial wall remodeling require the presence of the endothelium. These observations underscore the central importance of the endothelium in regulating the responsiveness of the arterial wall to flow.

4. Endothelial dysfunction and the development of atherosclerosis

Dysfunction of the arterial endothelium plays a critical role in a number of pathologies, most notably atherosclerosis. Atherosclerosis is an arterial disease whose pathological complications, namely heart attacks and strokes, are the leading cause of mortality in the Western world. The disease is characterized by the accumulation of lipids, proteins, and cellular components within the arterial wall. Over the years, our understanding of the events that trigger the onset of atherosclerosis and that govern the development and progression of the disease has evolved considerably. Early studies focused principally on the "lipid hypothesis" which postulated that atherosclerosis was attributable to the inability of the arterial wall to fully metabolize the lipids transported into it from the bloodstream, thereby resulting in the net accumulation of these lipids in the vessel wall [39]. Although this hypothesis is plausible and continues to guide many aspects of our understanding of the progression of atherosclerosis, it does not provide an explanation for the early events that trigger enhanced lipid transport. Because the endothelium is the primary barrier to macromolecular transport from the bloodstream into the arterial wall, subsequent efforts led to the "response to injury" hypothesis [40,41]. This hypothesis proposed that the earliest event in the development of atherosclerosis is endothelial injury that leads to EC denudation and thus exposes underlying connective tissue. In addition to enhanced macromolecular transport, platelets would adhere to the de-endothelialized arterial segment and release factors that stimulate the proliferation of smooth muscle cells, contributing to the development of early atherosclerotic lesions.

The "response to injury" hypothesis had to be modified when it was recognized that the endothelium remains intact in the early stages of atherosclerosis. We now recognize that true endothelial injury and desquamation does not generally occur during the development of atherosclerosis and that when it does, it occurs in the advanced stages of the disease. Therefore, it is now thought that the earliest event in the development of atherosclerosis is the "dysfunction" of the endothelium. The type of endothelial dysfunction most commonly implicated in atherogenesis (the onset of atherosclerosis) is cellular inflammation [42,43]. EC inflammation leads to an increase in vascular permeability, thus leading to enhanced macromolecular transport and accumulation within the arterial wall. EC inflammation is also associated with the increased expression of a host of surface adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and monocyte chemoattractant protein-1 (MCP-1), which increases leukocyte recruitment, adhesion, and transmigration. These leukocytes eventually become trapped within the lipid-laden arterial wall and ultimately become one of the principal components of advanced atherosclerotic plaques.

5. Role of blood flow in the development of atherosclerosis

A key feature of atherosclerosis is that early lesions are focal in nature, i.e. they do not develop randomly within the arterial tree but rather preferentially in regions of large arterial curvature and near arterial branches and bifurcations [44]. This observation suggests that there must be some localizing factor(s) for early disease development. Common risk factors for atherosclerosis that are used clinically include elevated cholesterol levels, hypertension, smoking, stress, diabetes, male gender, aging, and a sedentary lifestyle. None of these risk factors explains the focal nature of the disease. Because highly curved arterial segments and regions near branches and bifurcations are expected to be associated with "disturbed" flow patterns due to the complex arterial geometry in those regions, it has been suggested that disturbed blood flow promotes the development of atherosclerotic lesions and acts as a localizing factor for these lesions.

Two key questions regarding the involvement of disturbed flow in the localization of atherosclerosis are: (1) what does "disturbed" flow really mean? and (2) what aspects of flow disturbance correlate with the localization of lesions? These questions have motivated a large number of experimental and computational studies that have aimed to describe arterial fluid mechanics in detail and to relate particular fluid mechanical parameters to the topography of atherosclerotic lesions. This endeavor, of course, is quite complicated. The fluid mechanical problem is difficult because of the complex arterial geometry, the pulsatile nature of blood flow, the non-Newtonian behavior of blood, and the compliant nature of arteries. Correlating fluid mechanical features with the localization of lesions is complicated by differences in lesion localization among different animal models as well as the fact that lesions that protrude into the arterial lumen themselves perturb arterial flow patterns. Some of these notions are next described in more detail.

5.1. Arterial fluid mechanics

The past three decades have witnessed a large number of experimental and computational investigations of arterial flow fields. Because it appears logical that disturbed arterial blood flow participates in the development and progression

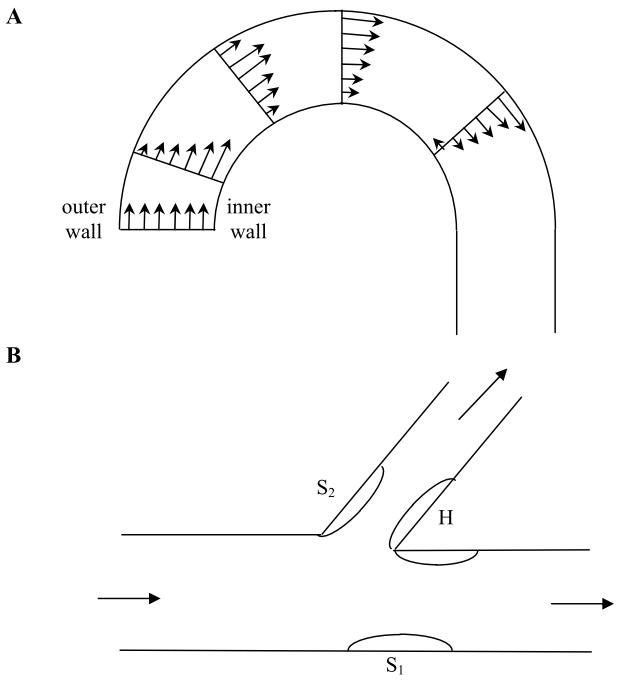

Fig. 3. General features of flow in arterial regions where atherosclerotic lesions are often observed. A: Steady flow in a curved vessel with a flat inlet velocity profile. In the vessel midplane, the velocity profile is initially skewed towards the inner wall, but this skewness is rapidly reversed to the outer wall. Depending on the Reynolds number and the extent of curvature, flow along the inner wall may separate. B: Flow in a branch or bifurcation. The outer walls experience lower wall shear stress than the inner walls. Depending on the flow conditions, flow at the outer walls may separate. S_1 and S_2 denote potential flow separation zones. H denotes regions of high wall shear stress.

of atherosclerosis through an effect on the arterial wall, most fluid mechanical studies have focused on wall shear stress distribution and to a lesser degree on the pressure distribution at the arterial wall. We simply highlight some of the major findings from these studies; an exhaustive review of the literature in this area is beyond the scope of the present article.

The earliest experimental studies were performed in glass or plastic models of arterial branches and bifurcations [45–50]. This was subsequently followed by flow visualization studies in casts of arterial segments and in excised arteries [51–56]. Advancements in magnetic resonance (MR) and Doppler ultrasound imaging technology subsequently made it possible to study arterial flow details in vivo at sufficiently high spatial and temporal resolution to extract useful information on the wall shear stress distribution [57–60].

In the case of computational investigations, early simulations were two-dimensional and limited to steady flow. However, rapid advances in computational methods and machine speed have enabled full unsteady flow simulations in three-dimensional geometries [61–68]. Recent computations have also modeled the fluid–structure interactions between blood flow and the compliant arterial wall in an effort to gain an appreciation for the effect of wall motion on the wall shear stress distribution [69–72].

What have we learned from the experimental and computational fluid mechanical studies alluded to above? Although these studies have been performed on different arterial sites including the thoracic and abdominal aorta, the iliac bifurcation, and the carotid and coronary arteries and although these different sites are characterized by considerably different geometries and flow conditions, some general features of the flow field at branches and bifurcations and in regions of sharp vessel curvature have emerged. More specifically, we now have some level of understanding of some general effects of arterial geometry, flow pulsatility, the non-Newtonian character of blood, and wall compliance on arterial blood flow patterns. It should be recognized, however, that these general features, which are described next and which are common to many of the arterial sites prone to the development of atherosclerosis, only take us so far because of the many fluid mechanical peculiarities of each arterial site.

5.1.1. Effect of arterial geometry

Arterial geometry is probably the single most important determinant of local blood flow patterns and of the likelihood of occurrence of arterial flow disturbance. As already mentioned, atherosclerotic lesions localize preferentially in arterial regions of sharp curvature as well as branching and bifurcation. Some general features of flow in these geometries are depicted schematically in Fig. 3. For steady flow through a curved vessel with a uniform inlet velocity profile, the wall shear stress is higher along the inner wall than along the outer wall near the entrance of the vessel, but this trend is reversed shortly thereafter due to flow acceleration at the outer wall (Fig. 3A) [73]. A prominent feature of flow in curved vessels is the presence of secondary flow which often takes the form of two counter-rotating Dean vortices [74–77]. When

combined with the primary flow, the flow structure consists of two forward-moving and counter-rotating helices. The most prominent example of vessel curvature in the arterial system is the aortic arch. The arch has branches emanating from its outer curvature, and these branches have a significant effect on the flow field in the arch. More specifically, it has been reported that in the aortic arch of both the dog and the rabbit, the presence of the branches modifies the secondary flow structure so that a single vortex (rather than two) is present [55,56]. Furthermore, studies suggest that there are large spatial gradients of wall shear stress at the entrance of the aortic arch branches [55,56,65].

If the vessel curvature in the aortic arch is sufficiently sharp and if the fluid has sufficient inertia, then the boundary layer separates at the inner vessel curvature (Fig. 3A). For two-dimensional flow, this separation zone is occupied by closed recirculating flow streamlines. For the more realistic case of three-dimensional flow, on the other hand, the recirculation zone is occupied by forward-moving flow streamlines that often follow complex helical paths [55,56].

Flow studies on branching and bifurcation geometries have revealed that the outer walls of these geometries typically experience lower wall shear stress levels than the inner walls (Fig. 3B). Depending on the bifurcation/branching angle, the flow Reynolds number, and the flow ratio between the daughter branches, flow in the lower shear stress zones along the outer walls may even separate. Here again, the flow separations zones are occupied by either recirculating flow streamlines or by forward-moving helical flow [52,56,64].

5.1.2. Effects of flow pulsatility and the non-Newtonian character of blood

The pulsatile nature of blood flow renders arterial flow fields highly dynamic with large temporal variations in wall shear stress and pressure. With pulsatility, the flow separation and recirculation zones described in the previous section periodically grow and shrink (or may even transiently appear and disappear). Consequently, these zones become associated with periodic directional oscillations in blood flow [78–80]. As will be described in more detail in a later section, these oscillations in blood flow have been suggested to correlate with the localization of atherosclerotic lesions.

At very low shear rates (below $\sim$100 s^{-1}), blood is a shear thinning fluid [81], but this non-Newtonian behavior disappears at higher shear rates. Therefore, the effect of the non-Newtonian behavior of blood on the near-wall flow field is most prominent in regions of very low wall shear rate, as is often the case in the flow separation and recirculation zones discussed above. In these zones, the non-Newtonian behavior of blood appears to reduce the size of the recirculation zones, and the extent of this reduction depends on the flow Reynolds and Strouhal numbers [80].

5.1.3. Effect of arterial wall compliance

The effect of wall compliance on arterial flow fields is a matter of controversy. Several studies have argued that the relatively small amplitude of wall motion (change in arterial cross-sectional area from peak systole to peak diastole of $\sim$5–7%) has a negligible effect on the flow field, and some studies of arterial wall shear stress profiles appear to support this conclusion. For example, it has been reported that the overall wall shear stress characteristics are not significantly altered by wall compliance for flow at intracranial aneurysms and in coronary artery bypass grafts [82,83]. On the other hand, other investigations suggest that the effect of wall compliance may not be at all negligible. For instance, in the coronary arteries, it has been suggested that wall compliance significantly reduces spatial gradients in wall shear stress [71]. In the carotid artery, wall motion appears to have little effect on the time-averaged wall shear stress while having a significant impact on the occurrence of flow reversal during the course of the cardiac cycle [72]. Therefore, the extent to which wall motion affects the local flow field appears to depend on the specific arterial site considered. There is a critical need for systematic investigations of how vessel geometry and flow conditions modulate the effects of wall compliance on arterial flow fields. It should also be noted that most studies of the effects of arterial wall compliance have considered purely elastic walls. Arterial walls exhibit non-linear mechanical behavior.

5.2. Relations between arterial fluid mechanics and atherosclerosis

There have been great efforts aimed at correlating particular patterns of wall shear stress obtained from experimental and computational fluid mechanical studies with the localization of early atherosclerotic lesions. The earliest such investigations suggested that lesions develop in arterial regions where the wall shear stress is particularly high [84]. The thinking was that a sufficiently high shear stress level would induce EC damage, thereby promoting the development of lesions. A competing theory that rapidly emerged, however, was that atherosclerosis develops in arterial regions subjected to particularly low levels of shear stress (below $\sim$0.5 Pa) [85,86]. The rationale was that low shear regions increase the residence time of macromolecules near the arterial wall, thus increasing mass transport into the wall and leading to progressive accumulation of deposits. For several years thereafter, the high vs. low shear stress controversy dominated the field. By the end of the 1970s, however, the low shear stress theory appeared to prevail for the following three reasons: (1) as already mentioned, a number of animal studies indicated that atherosclerosis in its early stages develops in the presence of an intact (undamaged) endothelium; (2) it was recognized that the shear stress levels required to denude arterial ECs ($>$35 Pa) were considerably higher than those present in vivo; and (3) based on the understanding of arterial fluid mechanics at branches and bifurcations available at the time, the topography of early atherosclerotic lesions in human cadavers appeared to correlate more closely with low shear regions than high shear regions (upstream of major aortic branches and in flow separation and recirculation zones). Thus, the low shear hypothesis rapidly became fairly widely accepted in the community.

The low-shear hypothesis had to be expanded when further fluid mechanical studies demonstrated that flow separation zones within which wall shear stress is low in the case of steady flow periodically grow and shrink when flow pulsatility is taken into account. Therefore, these regions are associated simultaneously with low time-averaged shear stress and periodic directional oscillations in shear stress. Thus, the low shear hypothesis of atherosclerosis evolved to the low and/or reversing shear hypothesis [78]. Although this remains the most widely cited correlation between hemodynamics and atherosclerosis, several studies in the past decade have raised questions about how predictive and general this association might be. For instance, recent experiments on rabbits suggest that lesion localization is more likely to correlate with high rather than low wall shear stress [87]. These conclusions are further complicated by the observation that the topography of atherosclerotic lesions in rabbits is in itself dynamic in time with lesion localization around aortic branches changing dramatically with animal age [88].

In addition to the hypotheses described above for correlations between particular fluid mechanical parameters and the incidence of atherosclerosis, several others can be found in the literature. Because studies on ECs in culture have revealed that cells respond not only to the shear stress level to which they are subjected but also to spatial and temporal gradients in shear stress [89,90], these parameters have also been proposed to play a role in the link between arterial flow and atherosclerosis. Finally, a recent study has implicated temporal oscillations in spatial wall shear stress gradients [91].

In summary, despite intensive investigation over the past four decades, a definitive correlation between arterial fluid mechanics and the localization of atherosclerotic lesions has remained elusive, a situation that can probably be attributed to a number of factors. One factor is that the same arterial location can be simultaneously subjected to several of the fluid mechanical parameters implicated in atherosclerosis. For instance, a flow recirculation zone is exposed to: (1) a low time-averaged wall shear stress, (2) spatial gradients of shear stress as the wall shear stress goes from zero at the flow separation and reattachment points to non-zero within and outside the recirculation bubbles, (3) periodic flow directional reversal as the recirculation zone repeatedly grows and shrinks with every cardiac cycle, and (4) large temporal gradients of wall shear stress during the course of the pulsatile cardiac cycle. A second factor that has prevented a consensus on the involvement of arterial flow in atherosclerosis is that different studies have been conducted on different vessels in different animal models. In principle, it is possible that correlations between flow and atherosclerosis, if they truly exist, may not be the same for all vessels or in different animals. A third factor, of course, is the possibility that there is no single hemodynamic parameter that correlates with the localization of atherosclerosis. Finally, there is evidence that there are significant differences in baseline wall shear stresses in arteries among different animal species [92]. Thus, what would be considered “low” wall shear stress in one species would in fact be a “high” level of shear stress in another species. An intriguing idea is that these differences in shear stress among species lead to a situation where the shear stress set-point for baseline EC function is different for different species so that flow-induced endothelial dysfunction would occur at different shear stress levels in different animal species.

6. Flow-induced biological responses in ECs

As already described, the development of atherosclerosis requires EC inflammation [42,43]. Therefore, regardless of the fluid mechanical parameters that best correlate with the localization of atherosclerosis, an understanding of the role of arterial flow in the development of atherosclerosis requires elucidating the effects of flow on EC function and dysfunction. This falls within the realm of cellular mechanotransduction, the field of study that aims to describe how cells sense mechanical stresses and how these stresses are subsequently transduced into biological/biochemical signals that regulate cellular structure and function.

Research over the past two decades has established that flow induces numerous humoral, metabolic, and structural responses in ECs [3–5,93]. The onset of steady flow elicits a number of very rapid responses including activation of integrins [94], stimulation of flow-sensitive ion channels [18,95,96], induction of the tyrosine kinase Src and of GTP-binding proteins (G proteins) [97,98], alteration in cell membrane fluidity [99,100], changes in intracellular pH [101,102], and the release of ATP [103]. Steady flow also induces release of nitric oxide, a powerful vasodilator whose production is generally considered to be protective against atherosclerosis [104,105]. These virtually immediate responses are rapidly followed by mobilization of intracellular calcium [106,107] and stimulation of mitogen-activated protein (MAP) kinases [108,109]. Activation of the transcription factors NF-κB and AP-1 rapidly follows [110], leading ultimately to changes in transcriptional and translational levels of a broad spectrum of adhesion molecules and growth factors [3,93,111]. Throughout this entire process, extensive but progressive cytoskeletal remodeling occurs, ultimately leading to cellular morphological changes so that ECs become elongated and aligned in the direction of the applied flow [112–114].

In vivo, all ECs are constantly exposed to flow; however, it is only cells that are subjected to “disturbed” flow that become dysfunctional and prone to the development of atherosclerosis. Thus, as mentioned in the Introduction section, an important concept in the field of endothelial mechanotransduction is “mechano-differentiation”, i.e. the ability of ECs to distinguish among and to respond differently to different types of flow and to different flow directions [115,116]. Although the mechanisms governing EC mechano-differentiation remain unknown, there are many examples of differential EC responsiveness to different types of flow. For instance, while steady flow induces a mix of genetic responses that leads to an anti-inflammatory and hence atheroprotective EC phenotype, purely oscillatory flow (i.e. oscillations about a zero mean flow; henceforth referred to as “oscillatory flow”) elicits a genetic response that leads to a cellular profile that is pro-inflammatory and atherogenic [43]. More specifically, prolonged exposure of ECs to relatively elevated levels of steady

shear stress (>1 Pa) down-regulates the expression of the pro-inflammatory molecules intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [117] and up-regulates the expression of the anti-inflammatory molecules interleukin-10 (IL-10) and transforming growth factor beta-1 (TGF-β1) [118,119]. Oscillatory shear stress, on the other hand, has the opposite effect on these molecules [14]. In addition to their different effects on gene expression, steady flow and oscillatory flow have different effects on a number of other flow responses in ECs including the mobilization of intracellular calcium [13] and the activation of flow-sensitive ion channels [16,17,120]. From a structural standpoint, steady flow induces extensive actin remodeling in ECs while oscillatory flow fails to elicit this cytoskeletal reorganization [12,15].

7. Mechanisms of EC responsiveness to flow

Despite the ever-expanding database on flow-induced biological responses in ECs, much remains unknown about the governing mechanisms. As already indicated, cellular responsiveness to flow can be thought of as a sequence of events involving sensing of the mechanical stimulus (mechanosensing), transmission of the mechanical signal to one or more intracellular target sites (mechanotransmission), and finally the transduction of the mechanical signal into a biochemical message that enables or catalyzes a biological/biochemical reaction (mechanotransduction). To these processes we add the mechanisms that govern cellular discrimination among different flow waveforms (mechano-differentiation) which, in principle, can occur either at the mechanosensing or mechanotransmission stage (or both). The following sections provide some ideas about the mechanisms that govern these various processes.

7.1. Mechanosensing

It appears logical to assume that mechanosensing in ECs is associated with some of the earliest responses to flow described above. It also appears reasonable to assume that mechanosensing is enabled by cell-surface or transmembrane structures that, due to their location, would be strategically positioned to sense extracellular forces and to rapidly convey the mechanical signals to the intracellular space. Therefore, structures that have been proposed as candidate mechanosensors include integrins [94], flow-sensitive ion channels [120], G protein-coupled receptors [98], and molecular complexes at cell–cell junctions [121].

Two other intriguing ideas have also been proposed. The first is the notion that the cell membrane itself (or specific membrane subdomains) can act as a mechanosensor. The idea is that mechanical stresses can alter the physical properties of the cell membrane and that these alterations can initiate biological responses via an effect on membrane-associated proteins. Support for this hypothesis is provided by experimental data demonstrating that flow increases EC membrane fluidity (i.e. decreases membrane viscosity) [99,100] which may increase the mobility of membrane-associated proteins and hence facilitate molecular interactions. Further evidence for a role for the cell membrane in mechanosensing comes from the observation that flow hydrolyzes guanosine triphosphate (GTP) encapsulated in lipid vesicles [122], demonstrating that flow is capable of inducing a biological response simply through a lipid bilayer and in the absence of transmembrane proteins, cytoskeletal elements, or intracellular organelles. Despite these interesting observations, it remains unknown how flow forces induce changes in cell membrane viscosity, whether or not flow-induced GTP hydrolysis in vesicles occurs as a direct result of flow-induced changes in vesicle membrane viscosity, and how directly applicable flow-induced GTP hydrolysis in vesicles might be to cells.

The second interesting mechanosensing idea that has generated considerable interest of late is the notion that the cellular glycocalyx acts as a mechanosensor in ECs as well as in other cell types [122–126]. The glycocalyx is a dense polymeric brush that lines the surfaces of many cell types including ECs. It is composed of various glycoproteins that bear acidic oligosaccharides and terminal sialic acids as well as proteoglycans that have a variety of glycosaminoglycan side chains including heparan sulfate, chondroitin/dermatan sulfate, and hyaluronic acid or hyaluronan [123]. The glycocalyx has been reported to have a height of 0.1–1 μm, quite a prominent structure in light of the fact that overall EC height is only 2–4 μm [19]. At its base, the glycocalyx has been suggested to traverse the cell membrane and to link directly to the intracellular actin cytoskeleton [123,125]. It has been argued that the presence of a thick glycocalyx on the cell surface greatly reduces flow velocity near the surface so that the magnitude of the wall shear stress due to physiological flows becomes negligible (i.e. practically zero). Thus, mechanotransduction would result not from the effect of flow on structures on the cell surface or on the cell membrane itself but rather from flow-derived forces on the tips of a rigid glycocalyx that generate significant moments on and hence deflections in intracellular actin filaments [123]. Support for the involvement of the glycocalyx in flow-mediated EC mechanotransduction is provided by data demonstrating that enzymatic digestion of specific constituents of the glycocalyx abolishes some (but not all) flow-induced biological responses [126]. On the other hand, data that suggest that the glycocalyx is far from a rigid structure [127] cast some doubt about the validity of the idea of a rigid glycocalyx that can transfer significant moments at its base. Therefore, more definitive answers about the mechanisms governing the involvement of the glycocalyx in mechanosensing await further information on the mechanical properties of the glycocalyx and its ability to support and transfer force.

In this section, we have referred to a number of candidate mechanosensors. It is of course possible (and even probable) that ECs do not possess a single mechanosensor. Rather, there may be various specialized mechanosensory complexes that provide a level of redundancy needed to ensure mechano-responsiveness and allow the wide spectrum of observed biolog-

ical responses to mechanical stimulation. How these various mechanosensors communicate with one another and integrate their actions to coordinate overall cellular mechanosensing is one of the principal challenges in this field.

7.2. Mechanotransmission

A key question in the field of cellular mechanotransduction is how flow signals are transmitted from the cell surface to the nucleus where they regulate gene expression and protein synthesis. Flow activates a number of transcription factors in ECs, which undoubtedly play an important role in regulating flow-induced changes in gene and protein expression. This can be viewed as a second messenger-mediated "biochemical" pathway that is governed by intracellular signaling cascades. This type of signaling is under intense investigation by numerous molecular biologists and will not be discussed here. There is mounting evidence, however, that mechanical forces at the EC surface are also transmitted to various intracellular sites directly via the cytoskeleton. Within this "biophysical" signaling construct, the cytoskeleton plays the role of "hard wiring" ECs and of providing direct physical links between structures at the EC membrane and those within the cytoplasmic space [3,8–11]. These links serve to distribute mechanical forces to various intracellular transduction sites including the nucleus.

Several experimental observations lend support for the idea of force transmission from the cell surface to the intracellular space via the cytoskeleton. For instance, binding of ECs to microbeads coated with extracellular matrix ligands for integrin receptors induces rapid formation of focal adhesion complexes that mediate the transfer of the mechanical stress to the internal cytoskeleton [8]. Mechanical tugging on these beads leads to nuclear deformation and elongation in the direction of the applied mechanical force [9]. In contrast, binding of beads coated with acetylated low density lipoprotein, a ligand for metabolic receptors that only physically connect to the submembranous cytoskeleton (as opposed to the internal cytoskeleton), does not support mechanical force transfer across the cell membrane, and mechanical tugging on these beads fails to induce nuclear deformation and elongation [9]. Mechanical tugging-induced deformation and elongation of the nucleus also occurs in the case of integrin-bound beads on ECs whose membranes and cytosolic components had been extracted (with Triton X-100), suggesting that force transmission from integrins to the nucleus occurs directly though the cytoskeletal lattice and not through more indirect pathways involving diffusion-based chemical signaling or protein polymerization [9]. Disruption of actin filaments with cytochalasin-D abolishes mechanical force-induced nuclear deformation and elongation [9], suggesting that an intact actin network is required for force transmission to the nucleus.

Additional support for the concept of direct force transmission via the cytoskeleton is provided by data demonstrating that applying a force to microbeads bound to integrins on cell surfaces leads to microtubule displacement at various discrete locations within the cells [7,128]. Importantly, the locations of microtubule displacement correspond to locations of force-induced activation of the tyrosine kinase Src [7], suggesting that Src activation may have occurred as a result of microtubule strain. Importantly, Src activation is observed at distances of $\sim$10 μm from the point of force application only 100–200 ms after the onset of force application [7]. Such rapid long-distance force transmission is observed neither in signaling cascades mediated by diffusion–reaction processes which typically require more than 10 s to travel the same distance nor in force propagation through the viscous cytoplasm where the applied force gets rapidly damped. The Src response is largely abolished when actin stress fibers are pharmacologically disrupted or when the molecular motors that generate tension within these stress fibers are disabled [7]. These findings lend additional support for the importance of the cytoskeleton, particularly actin stress fibers, in rapid long-distance force transmission and suggest that prestress (i.e. pre-existing tension) in the stress fibers plays a critical role in enabling rapid propagation of mechanical signals within cells.

A unique feature of mechanical force transmission via the cytoskeleton is that the filamentous nature of cytoskeletal architecture enables the spatially heterogeneous distribution of an applied mechanical force within cells [7,128,129], thereby allowing force focusing on specific intracellular sites where a particular biological effect is desired. In light of the myriad targets that cytoskeletal force distribution provides, the wide range of possible response time constants associated with these targets, and interactions among these various responses, force transmission via the cytoskeleton allows for a potentially very rich array of responses to mechanical stimuli exerted on the cell surface.

Despite the experimental evidence for rapid long-distance force transmission via the cytoskeleton in cells, the physical phenomena that govern the dynamics of this transmission remain unknown. To address this gap, a recent model has been developed to describe the spatiotemporal dynamics of force transmission via the cytoskeleton [130,131]. Because experimental data indicate a central role for actin stress fibers in force transmission via the cytoskeleton [7,11,128] as has already been mentioned, particular emphasis has been placed in the modeling on understanding the dynamics of force transmission through actin stress fibers. The model considered the highly simplified case of a single actin stress fiber that links an integrin on the cell surface directly to the nucleus as depicted in Fig. 4A and determined the time required for the effect of a mechanical stress applied at a particular location along the stress fiber to propagate to the nucleus. In the analysis, the stress fiber was assumed to be a viscoelastic material whose constitutive relation is governed by the Kelvin–Voigt model (a spring in parallel with a dashpot) in accordance with experimental observations derived from laser severing of individual stress fibers [28]. The model also accounted for a constant level of prestress in the stress fiber and included external damping of stress fiber motion due to cytosolic drag (assuming Stokes flow). Both constant and oscillating stresses applied to the stress fiber were considered.

The model results demonstrated that for the case of a constant stress, mechanical signals applied orthogonal to the stress fiber propagate to the nucleus much more rapidly than signals applied in the axial direction. More specifically, orthogonal stresses propagate a distance of $\sim$10 μm in $\sim$10 ms, whereas an axial stress requires $\sim$10 s to travel the same distance as

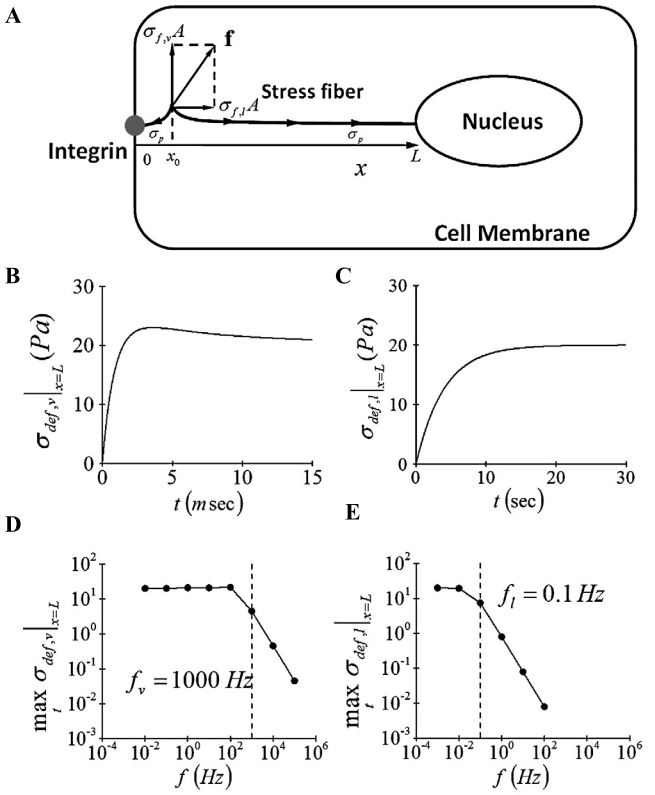

Fig. 4. A. Schematic diagram of the actin stress fiber model. B and C. Temporal evolution of the transverse (panel B) and axial (panel C) deformation-related stress at the nucleus ($x = L$) with steady forcing. D and E. Effect of the forcing frequency on the amplitude of transverse (panel D) and axial (panel E) deformation-related stress at the nucleus ($x = L$) for the case of oscillatory forcing. Note significant stress damping at higher frequencies.

depicted in Figs. 4B and C [130,131]. Thus, only forces applied orthogonal to stress fibers are able to propagate at speeds comparable to those observed experimentally [7], suggesting that rapid long-distance force transmission is only possible in cells whose stress fibers are aligned virtually parallel to one another and oriented orthogonal to the applied force. The model results also demonstrated that: (1) rapid long-distance force transmission in cells is driven primarily by prestress in the actin stress fibers, (2) stress propagation velocities are limited principally by the internal (or material) viscosity of the viscoelastic stress fibers, and (3) the effects of cytosolic damping are largely negligible [130]. It should be mentioned that in this model, mechanical signal transmission dynamics are derived from the deformation-related stresses within the actin stress fiber.

In the case of oscillatory forcing applied to the stress fiber, the amplitude of the deformation-related stress felt at the nucleus is not surprisingly a strong function of the forcing frequency. Figs. 4D and E illustrate the peak amplitude of the deformation-related stress at the nucleus as a function of forcing frequency for transverse and axial forcing, respectively. In both the axial and transverse directions, a low-frequency (<0.1 Hz) mechanical stimulus is transmitted to the nucleus without decay in its amplitude, whereas a high-frequency mechanical stimulus undergoes significant decay in amplitude. This implies that individual stress fibers act as low-pass filters of mechanical forcing. Interestingly, transverse motion exhibits a much broader filter width than axial motion: the filter width for transverse motion extends to $f \sim 1000$ Hz whereas the one for axial motion extends to only $f \sim 1$ Hz.

The experiments and models of force transmission from the cell surface to the nucleus via cytoskeletal connections inherently assume that the cytoskeleton links directly to the nucleus. However, research over the past decade has demonstrated that links between the cytoskeleton and the nucleus are not direct but are rather provided by specialized linker proteins such as the nesprin proteins. In ECs, the nesprin protein family contains at least three members: nesprin-1, -2, and -3. As schematically depicted in Fig. 5, nesprin-1 (for nuclear envelope spectrin-1; also known as syne-1, myne-1 or enaptin) and nesprin-2 (also known as syne-2, myne-2 or NUANCE) connect the actin cytoskeleton to the nuclear envelope [132–137], whereas nesprin-3 connects the intermediate filament cytoskeleton to the nuclear envelope. Nesprin-1 and -2 have the following features in common: (1) they are very large (>800 kD) – nesprin-1 is over 8200 amino acids in length and nesprin-2 is over 6800 amino acids long. (2) Their N-termini have two calponin-like domains that bind actin. (3) Their C-termini contain KASH (Klarsicht, ANC-1, and syne homology) domains that are sufficient for outer nuclear membrane targeting. (4) The region between the two termini consists of spectrin repeats that align end-to-end to form rope-like structures. Nesprin-3 is a much smaller protein than the other nesprins (~108 kD). Similar to nesprin-1 and -2, nesprin-3 is a transmembrane protein of the outer nuclear membrane and contains a C-terminal KASH domain; however, unlike the other two nesprins, it lacks an actin-binding domain and binds instead to plectin which associates with intermediate filaments [138].

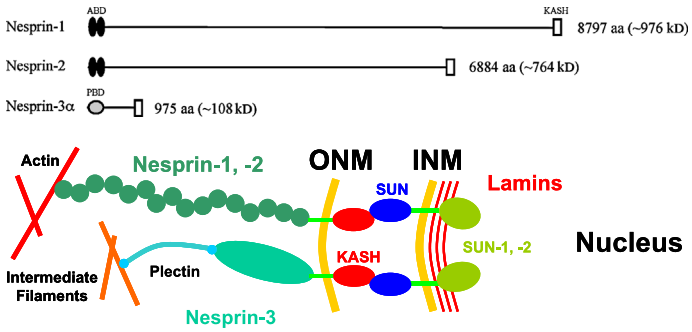

Fig. 5. Three types of nesprin proteins are known to be present in ECs: nesprin-1, -2 which connect the actin cytoskeleton to the nucleus and nesprin-3 which connects the intermediate filament cytoskeleton to the nucleus. All nesprins have a KASH domain at one end that allows targeting to the nucleus. At the other end, nesprin-1 and -2 have an actin-binding domain (ABD) whereas nesprin-3 has a plectin-binding domain (BDP) that provides the link to intermediate filaments. Nesprin-1 and -2 are very large (~976 kD and ~764 kD, respectively), whereas nesprin-3 is much smaller (~108 kD). In the region between the outer nuclear membrane (ONM) and the inner nuclear membrane (INM), nesprin proteins bind to SUN proteins which, in turn, bind to nucleoskeletal proteins such as the lamins. The integrin–cytoskeleton–nesprin–SUN–lamin sequence constitutes a continuous pathway that physically connects the cellular extracellular matrix to the inside of the nucleus. Color available online.

Beyond connecting the cytoskeleton to the nucleus, the C-terminal KASH domain of nesprin proteins also binds to the inner nuclear membrane proteins SUN-1 and SUN-2 which associate with various nucleoplasmic proteins including lamin A/B, emerin, and even possibly chromatin [11,139] (Fig. 5). Importantly, these various connections provide a direct physical link between the cellular cytoskeleton and the nucleoskeleton, potentially providing a direct pathway for mechanical force transmission into the nucleus. The role of nucleoskeletal proteins in modulating EC cellular responses to flow remains to be elucidated.

The role that nesprins play in regulating cellular function remains poorly understood and is under intense investigation by several groups. Nesprin-1 and -2 play an important role in nuclear anchoring and positioning [135,137,140]. Direct demonstration of the involvement of nesprin-1 in nuclear positioning has been provided in a transgenic mouse model where the conserved C-terminal KASH domain of nesprins was over-expressed and acted in a dominant interfering manner, displacing endogenous nesprin from the nuclear membrane [140]. In this model, muscle nuclei failed to aggregate at the neuromuscular junction, indicating that localization and anchoring of synaptic nuclei require nesprin proteins. In ECs, nesprin-3 regulates cell morphology, with nesprin-3 expression knockdown leading to prominent cellular elongation [141]. It also modulates perinuclear cytoskeletal organization as well as the connectivity between the nucleus and the centrosome [141]. More recently, nesprin-3 expression knockdown has been shown to alter aspects of microtubule dynamics in ECs even far away from the nucleus [142]. This is an intriguing finding in light of the fact that nesprin-3 is thought to link to the intermediate filament cytoskeleton (rather than to microtubules) and may point to previously unknown interactions between the two cytoskeletal filament networks. It is also an important finding because it suggests that nuclear membrane proteins may act as scaffolds that anchor cytoskeletal filaments and regulate the dynamics of these filaments over long distances within cells.

The role that nesprin proteins play in cellular mechanotransduction has only begun to be studied. Recent papers have shown that nesprin-1 modulates stretch-induced reorientation in ECs as well as stretch-induced nuclear rotation in fibroblasts [143,144]. Other recent data on ECs show that nesprin proteins regulate the transmission to the nucleus of forces applied to the cytoskeleton [145] and that nesprin-3 expression knockdown regulates flow-induced cellular polarization and migration [141]. How nesprins regulate EC mechanotransduction remains unknown but will undoubtedly be the focus of future studies.

7.3. Mechano-differentiation at the levels of mechanosensing and mechanotransmission

As already described, ECs are able to distinguish among and respond differently to different types of flow; however, the mechanisms governing EC mechano-differentiation remain poorly understood. Conceptually, mechano-differentiation may occur at either the level of mechanosensing or mechanotransmission. Studies on flow-activated ion channels, which are candidate mechanosensors in ECs, have demonstrated that while steady flow activates both flow-sensitive K^+ and Cl^- channels, 1-Hz oscillatory flow activates K^+ channels but not Cl^- channels [16,17]. Consequently, steady flow and oscillatory flow have different effects on cell membrane potential [16] and hence presumably on intracellular ionic composition. This has been proposed as one possible mechanism by which ECs are able to sense differences between steady and oscillatory flow waveforms [120]. This can be thought of as an example of mechano-differentiation at the level of mechanosensing. Mechano-differentiation may also be possible at the level of mechanotransmission. The results of the model of force transmission via the cytoskeleton described above indicate that while a constant stress gets fully transmitted to the nucleus, an oscillatory axial stress in the physiological frequency range (0.1–10 Hz) would be significantly damped (cf.: Figs. 4D and E) and may therefore fail to be felt at the nucleus. This would provide a mechanism for an EC to distinguish between a steady flow signal and an oscillatory flow signal.

7.4. Mechanotransduction

After a mechanical signal is sensed at the cell surface and transmitted via the cytoskeleton to its target site, the signal needs to ultimately be transduced into a biochemical message in order to affect a biological response. How this transduction occurs in ECs remains poorly understood. It has been suggested that mechanical deformations at the target sites can induce conformational changes that lead to activation of specific molecules [146]. There is mounting experimental evidence that this may indeed be the case. For instance, mechanical stretching of cells has been shown to induce conformational changes in the focal adhesion protein vinculin in such a way to uncover cryptic binding sites to which talin binds and activates integrins [147,148]. Another direct example is the tyrosine phosphorylation of PECAM-1 in response to mechanical deformation [149]. These examples provide the basis for a general paradigm that may be applicable in many other cases.

8. Concluding remarks and future perspectives

The past two decades have witnessed a revolution in our understanding of flow-mediated mechanotransduction in vascular ECs and the implications of mechano-responsiveness to the development of atherosclerosis. Despite these advances, numerous questions in this field remain unanswered. Some of these questions fall in the realm of physics or mechanics, others are purely biological, and yet others require the coordinated integration of physics and biology. In the following, some key questions that are expected to receive considerable attention in this field in upcoming years are outlined, and perspectives on research directions and methodologies for addressing these questions are presented.

A key question in our pursuit of connections between arterial blood flow patterns and atherosclerosis is whether or not there are specific fluid mechanical parameters that truly correlate with the localization of atherosclerotic lesions. As described in the present review, many different parameters have been proposed, but none appears to be universally effective in predicting lesion topography. Part of the difficulty stems from the fact that the literature in this area is based on studies on different animal models; both the nature and localization of lesions are different for different animal models. Further complicating this picture is data demonstrating that lesion topography in an animal can vary considerably with age [88]. If particular fluid mechanical phenomena are responsible for lesion localization, then the dependence of lesion localization on age suggests the flow field changes fundamentally with age. Alternatively, if the flow field does not change significantly with age, then changes in lesion topography with age may indicate that the fluid mechanical parameters that regulate lesion localization change with age or that other age-related factors combine with fluid mechanical factors to determine lesion localization. These various concepts underscore the need for better understanding of arterial flow fields and a systematic investigation of the possible dependence of these flow fields on animal age.

In the field of mechanical force transmission in ECs, a particularly exciting concept is the emerging role of nucleus–bound proteins such as the nesprin family. As described above, our understanding of the role of these proteins in mechanotransmission remains at a very early stage. A critical issue that needs to be investigated is whether or not the physical connections that nesprins make with nucleoskeletal proteins play a role in regulating gene expression and protein synthesis and if so, what the relevant connections might be and what may be their mechanisms of action. A particularly intriguing related observation is the regulation by nesprins of microtubule behavior very far from the nucleus (at the EC periphery). This observation suggests a new paradigm whereby the nucleus is not simply a large reception center in a cell but is rather also a coordination center that acts as a scaffold that organizes cytoskeletal architecture throughout the cell. If experimentally validated, this notion promises to fundamentally change our understanding of the role of the nucleus in regulating cellular function in general and mechanotransduction in particular.

This review has discussed the notion of mechanical signal transmission via the cytoskeleton. Much remains to be discovered in this area. Research to date has primarily implicated actin stress fibers in force transmission. A critical question is whether or not microtubules and intermediate filaments also play a role in force transmission and if so, how. As alluded to earlier, mathematical models have been formulated to describe the physical phenomena that govern force transmission through a viscoelastic actin stress fiber; however, stress fibers in cells form networks with a variety of possible topologies. It is important to generalize the models to describe force transmission in representative networks of stress fibers. Furthermore, it would be interesting to formulate similar models for microtubules and intermediate filaments. However, these models have to take into account a number of issues. For instance, whereas immunofluorescent staining of stress fibers shows that they are generally very straight inside cells, staining of microtubules often shows them to be curved [150]. The dynamics of force propagation in curved structures may be significantly different from those in straight structures; therefore, microtubule behavior may be significantly different from that of stress fibers. In the case of intermediate filaments, the short persistence length suggests that thermal effects cannot be ignored. Therefore, the formulation in that case needs to account for these effects. In any case, a key need in this regard is a more complete and quantitative description of the three-dimensional architecture of the cytoskeleton and determination of the extent and nature of interaction among the three primary cytoskeletal filament networks in ECs. The lack of detailed information in this regard is a principal bottleneck for the development of more realistic models of cytoskeleton-mediated force transmission.

The past two decades have certainly witnessed the development of powerful tools to probe mechanotransduction in live cells. These tools include microfluidic platforms, fluorescent resonance energy transfer (FRET) probes, single molecule capabilities, nano-scale surface patterning technologies, improved microscopy and imaging tools, and more targeted and versatile molecular biological probes and techniques. Despite these advances, there is great need for expanding the toolbox.

Currently it is difficult to measure responses on a time scale faster than a few hundred milliseconds. Mathematical models of mechanotransmission predict that signals can propagate through stress fibers in less than 10 ms. Furthermore, the discrete nature of the cytoskeleton suggests that force transmission via stress fibers can occur in a highly spatially targeted manner. Testing the validity of the predictions of the models awaits the development of faster and more spatially resolved experimental capabilities.

In this review, we have focused primarily on the effects of flow on ECs. There is mounting evidence that biophysical factors due to the roughness and rigidity of the substrate on which cells are cultured also exquisitely regulate cellular structure and function [1,2]. In vivo, ECs are expected to be subjected to both flow forces on their apical surface and substrate forces on their basal surface. How these multiple signals are integrated within ECs and the nature of the resulting responses is an area that certainly merits investigation. A recent study in this direction suggests that depending on substrate features, either flow or substrate cues may constitute the dominant driver of EC elongation and alignment [151]. In addition to the biophysical stresses, ECs in vivo are constantly subjected to biochemical signals from smooth muscle cells as well as blood cells; therefore, determining how ECs interpret the combined effects of biophysical and biochemical stimulation is a worthwhile undertaking.

The vast majority of cellular mechanotransduction studies have been performed on cultured cells in vitro. There is a critical need for establishing whether or not similar responses are observed in vivo. This is obviously a very challenging endeavor. The ability to control flow in the microvasculature of live animals and to probe specific biological responses including microvascular permeability, intracellular calcium levels has been demonstrated [152,153] as have in vivo studies of the glycocalyx in microvessels [154]. Such studies open the door for future investigations of mechanotransduction events in vivo. However, access to the microvasculature is considerably easier than to the medium and large arteries in which atherosclerosis develops; therefore, new tools need to be developed in order to provide the capability of monitoring flow-induced EC responses in larger arteries in vivo. At the point when they become available, these tools promise to provide revolutionary insight into the role of arterial flow in the development and progression of atherosclerotic lesions.

Although the present paper has focused primarily on the role that perturbations in arterial flow and consequent endothelial dysfunction play in the development of atherosclerosis, there are other pathologies that involve these same factors. A notable example is the initiation and progression of aneurysms where disturbances in wall shear stress and associated endothelial dysfunction are thought to play a critical role [155]. Despite differences in the mechanisms governing the development of atherosclerotic lesions and aneurysms, knowledge gained from studies on one pathology can potentially provide important insight into the other. More generally, concerted interactions among scientists studying different but related pathologies promise to provide a unique perspective on the evolution of these pathologies and on the development of innovative therapeutic approaches to combat them.

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