Biophysics

Volume 2, Issue 3, 318-335.
DOI: 10.3934/biophy.2015.3.318
Received date 18 April 2015,
Accepted date 30 July 2015,
Published date 11 August 2015

http://www.aimspress.com/

Review

The Contribution of Cell Surface Components to the Neutrophil Mechanosensitivity to Shear Stresses

Michael L. Akenhead and Hainsworth Y. Shin *

Department of Biomedical Engineering, University of Kentucky, 143 Graham Avenue, Lexington, KY 40506, USA

* **Correspondence:** Email: hy.shin@uky.edu; Tel: +1-859-257-3783; Fax: +1-859-257-1856.

Abstract: This review discusses the regulation of neutrophils by fluid shear stress in the context of factors that may govern cell mechanosensitivity and its influence on cell functions. There is substantial evidence that mechanoreceptors located on the peripheral membrane contribute to the ability of shear stress to regulate cell activity. In the case of neutrophils, the formyl peptide receptor (FPR) and the CD18 integrins on the cell membrane have been shown to provide neutrophils with the ability to sense shear stresses in their local environment and alter their physiological state, accordingly. This configuration is also found for other types of cells, although they involve different cell-specific mechanoreceptors. Moreover, from an examination of the neutrophil mechanotransducing capacity, it is apparent that cellular mechanosensitivity depends on a number of factors that, if altered, contribute to dysregulation and ultimately pathophysiology. To exemplify this, we first describe the neutrophil responses to shear exposure. We then review two neutrophil mechanoreceptors, specifically FPR and CD18 integrins, which participate in controlling cell activity levels under physiological conditions. Next, we discuss the various factors that may alter neutrophil mechanosensitivity to shear stress and how these may underlie the circulatory pathobiology of two cardiovascular disease states: hypertension and hypercholesterolemia. Based on the material presented, it is conceivable that cell mechanosensitivity is a powerful global metric that permits a more efficient approach to understanding the contribution of mechanobiology to physiology and to disease processes.

Keywords: mechanotransduction; mechanobiology; circulation; fluid flow; leukocyte

1. Introduction

Neutrophils are among the most numerous of all leukocytes (i.e., white blood cells) in the blood. Due to their high sensitivity to pathogenic stimuli and their vast destructive potential, tight regulation of neutrophil activity levels in the circulation is essential for maintaining blood in a physiological, non-inflamed state. Failure to do so promotes a continuous state of cell activation that drives downstream organ injury [1]. This may be in the form of dysregulated release of proteases and cytokines into the vasculature that ultimately damages host tissues (i.e., a form of autodigestion) [2]. Alternatively, sustained neutrophil activation in the blood may indirectly contribute to tissue damage by interfering with tissue perfusion through an impact on blood flow regulation. It may also affect the flow behavior of blood (i.e., rheology) in the microcirculation [3]. This rheological effect couples the changes in the biological (i.e., morphological and surface adhesive) properties of activated neutrophils to their viscous flow behavior in microvessels, which may have a detrimental impact on hemodynamic resistance in the peripheral circulation [4,5]. In these ways, neutrophils may promote tissue injury that feeds a chronic inflammatory state in the circulation, a condition that has been recognized as a common denominator for many lethal human pathologies (e.g. cardiovascular disease, cancer, diabetes, Alzheimer's, etc.) [6,7].

Although neutrophil inactivity/quiescence in the circulation is predicated on the absence of inflammatory agonists, cellular and molecular mechanisms exist to proactively ensure leukocytes, including neutrophils, remain deactivated under physiological conditions (i.e., in the absence of acute inflammatory stimuli) [8,9]. This includes a number of mechanisms involving release of anti-inflammatory mediators (e.g., interleukin-10, transforming growth factor-β, cyclooxygenase-2, nitric oxide) by endothelial cells [10–12]. These mediators act by either downregulating inflammatory cytokine secretion or preventing pro-inflammatory receptors from binding to their ligands. Notably, some of these involve endothelial mechanotransduction of hemodynamic shear stresses that control the release of molecules (e.g., nitric oxide) that prevent platelet and neutrophil activity (see reviews [13,14]).

Over the past decade, we have contributed to a growing body of evidence [15,16] pointing to shear stress mechanotransduction by neutrophils as also being anti-inflammatory. During this time, we revealed new insights regarding how neutrophils respond to mechanical stresses in their local microscale environment and what factors influence this mechanoreception in health and disease. We also experienced a transition from using a reductionist viewpoint aimed at characterizing the shear stress responses of neutrophils to adopting a more global perspective to understand how the overall responsiveness of these cells to flow stimulation may play a role in vascular physiology and pathogenesis. What we have learned, thus far, is similar to that which has already been described; specifically, mechanotransduction is a complex process involving multiple pathways that converge upon a change (or changes) in cell activity. But the results of our efforts [17,18], and others [15,19,20], also suggest another concept; namely, there must exist some metric (e.g., mechanosensitivity) of the degree to which a cell's activity is altered by mechanical stresses.

In this review, we use the anti-inflammatory control of neutrophils by shear stress as the backdrop for discussing cell mechanosensitivity. Mechanosensitive events on the cell surface appear to be responsible for the ability of shear stress to restrict neutrophil activity by modulating intracellular signaling and/or extracellular events (e.g., adhesion receptor expression). Impairment of neutrophil mechanosensitivity is expected to result in tissue damage linked with reduced

microvascular perfusion. This review will first describe neutrophil shear responses. We will then focus on some key mechanosensitive receptors/molecules on the cell membrane that participate in controlling neutrophil activity, followed by a description of the factors that may alter neutrophil mechanoregulation at the cell surface. Finally, we discuss how impaired neutrophil shear sensitivity may arise and promote pathobiology using two clinical scenarios: hypertension and hypercholesterolemia.

2. Shear Stress Responses of Neutrophils

In general, neutrophil mechanoregulation by fluid flow has largely been studied in context of their central role in acute inflammation. In this regard, neutrophils exhibit a number of responses to fluid flow-derived shear stresses of 0–18 dyn/cm^2 [21–23] (Table 1), which is the typical range reported for the post-capillary venules [21]. Depending on vessel size and leukocyte position in the bloodstream (whether on the vessel wall or suspended in the lumen), it is possible for neutrophils to experience this shear range in any blood vessel. Notably, neutrophils may be exposed to hemodynamic shear stresses that can reach up to 95 dynes/cm^2 in some blood vessels [24]. However, since neutrophil recruitment during inflammation is generally limited to the post-capillary venules [25], the majority of studies, to date, have focused on neutrophil responses to shear stresses present within these microvessels. Notably, venular shear stresses are far below the >150 dynes/cm^2 shear magnitudes that have been shown to induce mechanical trauma to neutrophils [26].

Table 1. Effects of fluid flow on neutrophils.

Neutrophil Shear Response	Reference
Pseudopod retraction	[31]
F-actin depolymerization	[32]
CD18 cleavage	[18,30]
FPR internalization	[19,87]
L-selectin shedding	[33]
Reduced phagocytosis	[32]
Apoptosis	[34]

The principal evidence of neutrophils requiring a flow environment to remain deactivated under non-inflamed or mildly stimulatory conditions is that fluid stasis activates them. Neutrophils deposited on glass substrates [27], even in the absence of inflammatory agonists [28], activate as evidenced by their projection of pseudopods and adherence to the underlying surface. Furthermore, there is a lower percentage of activated cells (based on their morphology and surface expression of select adhesion molecules) in a suspension of neutrophils after exposure to flow in a near-constant 5 −10 dyn/cm^2 shear stress field of a cone-plate viscometer, in comparison to those of cell populations maintained under no-flow, but otherwise similar, experimental conditions [29]. It has also been shown, by intravital microscopic examination of the microvasculature of murine cremaster and rat mesentery, that leukocytes in venules sediment to vascular walls, project pseudopods, and adhere to

endothelium in response to upstream vessel occlusion when blood flow is stopped [15,30,31]. Upon reintroduction of blood flow, the neutrophils detach into the flow field in a mechanobiological, and not a physical shearing, fashion.

One of the more overt manifestations of the neutrophil mechanosensitivity to shear stress is its morphological impact, particularly its effect on pseudopod activity. This particular response represents one of the more direct evidence that shear stress promotes neutrophil deactivation. Specifically, neutrophils migrating on glass substrates, in the absence of agonist stimulation, retract existing cytoskeletal F-actin enriched pseudopodia and adopt a rounded morphology within 1–2 min of the onset of exposure to a non-uniform flow field (τ_{max}: 1–2 dyn/cm^2) imposed by a micropipette (tip diameter of 4–8 μm) [31]. Moreover, shear stresses, in the range of 1 - 10 dyn/cm^2 (typical of the circulation), elicit other effects consistent with neutrophil deactivation including depolymerization of the F-actin cytoskeleton and proteolytic cleavage of CD18 integrins (β_2 family of integrins involved in cell-cell adhesion) [30,31]. These data fall in line with the likelihood that pseudopod retraction involves disassembly of the underlying F-actin network and rapid disengagement of cell adhesion molecules that support and anchor these cellular projections to the underlying surface. Additionally, exposure to physiologic shear stresses inhibits phagocytosis by neutrophils, which involves pseudopods [32]. Shear stress exposure has also been demonstrated to induce shedding of L-selectin, which may serve to minimize neutrophil rolling under flow [33]. Finally, another cell-deactivating effect of shear stress is its upregulation of caspase 3-related apoptosis [34], which is in line with the short lifespan (i.e., 18–24 hours) of neutrophils passively circulating in the blood.

The cell-deactivating effects of shear stress mechanotransduction are consistent with it being an anti-inflammatory mediator of neutrophil activity. Evidence of this comes from in vitro and in vivo studies demonstrating that while low levels of cell agonists, such as fMLP (<1 μM) or platelet-activating factor (PAF, <10 μM) mildly stimulate neutrophils by promoting pseudopod formation, they do not compromise the ability of the cells to retract pseudopods in response to shear stress [27,30,31,35,36]. On the other hand, these same agonists above threshold levels (e.g., 1 μM fMLP, 10 μM PAF), which we believe demarcates an acute inflammatory scenario, abolish shear-induced pseudopod retraction [30]. Recently, we also showed a similar relationship between fMLP concentration and shear-related cleavage of CD18 integrins by HL60-derived neutrophil-like cells. Specifically, HL60 neutrophil-like cells exposed to shear in the presence of fMLP at concentrations >10 μM display an impaired ability to shed CD18 integrins [37]. Intuitively, this is reasonable since the blood biochemistry should be expected to override shear-induced deactivation of neutrophils when their heightened activity levels are required, such as during acute inflammation. Taken together, the accumulated evidence, to date, suggests that the shear stress regulation of neutrophils under physiological conditions is anti-inflammatory.

A critical issue regards how hemodynamic shear stresses in the blood microenvironment are sensed by the neutrophils. Conceivably, initiation of mechanotransduction occurs at the interface between the cell interior and extracellular milieu (i.e., the cell membrane). Along this line, biological molecules on the cell surface appear to be responsible for the ability of shear stress to modulate cell activity. In effect, transmembrane proteins, while serving as receptors for autocrine and paracrine signaling molecules, also appear to "moonlight" as mechanosensors of shear stress. This is the subject of the next section.

3. Shear Stress Sensitive Receptors Involved in Neutrophil Mechanoregulation

An important aspect of neutrophil regulation by physiological shear stresses is that they affect the cell without inducing any passive cell deformation [38]. In other words, there must be cell surface components that initiate signaling pathways arising from mechanical stress perturbations in the extracellular milieu. Focal adhesion complexes (FACs) and intercellular junctional complexes have been widely reported to serve as sites of mechanotransduction for many cell types, including leukocytes [39–42]. The regulation of cell activity by substrate tensile stresses and matrix stiffness [43] has been largely attributed to these mechanosensory complexes. However, it is important to emphasize that neutrophils respond to shear stress with reduced pseudopod activity and CD18 surface expression, whether they are adhered to/migrating on a surface or freely suspended in solution [30,31,44].

Additionally, neutrophils retract pseudopods independently of the spatial distribution of shear stresses imposed on their peripheral membrane by micropipette flow [31]. This was further supported in a later study that combined computational fluid mechanics with confocal microscopy of HL60-derived neutrophil-like cells retracting pseudopods in response to 2.2 dynes/cm^2 shear stress exposure in a parallel plate flow chamber [45]. In this study, HL60 neutrophil-like cells retracted pseudopods independent of their orientation with respect to the long axis of the imposed flow field and the distribution of both normal and shear stress components that developed on the membrane. Along with these data, the cell surface has been implicated in shear stress mechanotransduction due to its strategic location between the intracellular and extracellular milieu as well as its cell-specific composition of receptors, signaling molecules, and second messengers [46–48].

Conceivably, the cell membrane, itself, is a neutrophil mechanotransducer of shear stress considering the peripheral bilayer orchestrates the cyclical projection and retraction of pseudopods and uropods by influencing adhesion receptor function (such as CD18), ion transport (Ca^{2+}, etc.), and cytoskeletal dynamics [49]. Interestingly, shear stress exposure has been shown to alter the fluidity of the cell membrane [48,50]. This may be one way by which neutrophils sense shear stress, because changes in membrane fluidity influence neutrophil phagocytosis [51,52] and migration by modulating chemokine receptor number and affinity [53,54]. Like other cells, neutrophils may also transduce shear stress through lipid rafts [55-57]. This is possible considering lipid rafts regulate neutrophil signal transduction (chemokine-induced calcium flux, cell polarization, migration, integrin expression, and actin dynamics, etc.) and functions (shape change, motility, etc.) [46,58,59], as well as CD18-mediated T-cell adhesion [60] and neutrophil adherence [61]. Thus, shear stress may modulate neutrophil activity via membrane mechanotransduction.

However, the concept that the membrane serves as a mechanosensor lacks the cell-type specificity associated with shear stress mechanotransduction. This level of specificity likely depends on the peripheral membrane's enriched content of cell-specific and highly selective receptors and signaling molecules, some of which may be mechanosensitive. Many cell membrane associated proteins (Table 2) including various G protein-coupled receptors (GPCRs) [19,62-66], receptor tyrosine kinases (RTKs) [67–73], cell-cell adhesion molecules [39], ion channels [74–76], integrins [41,42,77–79], and signaling proteins (such as G-proteins [80,81]) have been implicated as shear stress sensors for various cells (e.g., endothelial cells, osteoblasts).

Table 2. List of cell-specific mechanoreceptors.

Mechanoreceptor	Receptor Type	Example Cell	References
Formyl peptide receptor (FPR)	GPCR	Leukocytes	[19]
CD18 integrins	Integrin	Leukocytes	[18,30]
L-selectin	Selectin	Leukocytes	[33]
Bradykinin B_2 receptor	GPCR	Endothelial cells	[63]
Vascular endothelial growth factor receptor 2	RTK	Endothelial cells	[67]
Tie2 receptor	RTK	Endothelial cells	[70]
PECAM-1/VE-cadherin	Junctional complex	Endothelial cells	[39]
Endothelin B receptor	GPCR	Smooth muscle cells	[62]
Epidermal growth factor receptor	RTK	Smooth muscle cells	[68]
Platelet-derived growth factor receptor-beta	RTK	Smooth muscle cells	[72]
Angiotensin II type 1 receptor	GPCR	Cardiac muscle cells	[64,66]
Type 1 parathyroid hormone receptor	GPCR	Osteoblasts	[65]
β_1 integrins	Integrin	Various cells	[77,78,79]
Shaker-IR	Voltage-gated K^+ channel	Transfected oocytes	[74]
TREK-1	Mechano-gated K^+ channel	Fibroblasts	[75]

For neutrophils, two cell surface receptors have been identified as putative mechanosensitive proteins that significantly contribute to the cell-deactivating effects of shear stress exposure; specifically, the formyl peptide receptor (FPR) and CD18 integrins.

3.1. Formyl peptide receptor (FPR)

FPR belongs to a group of cytokine-related GPCRs that modulate neutrophil activation, adhesion, and migration in response to chemotactic stimulation [82]. Makino et al. reported the initial evidence linking shear stress-induced pseudopod retraction and flow-mediated GPCR activity [15,19]. Specifically, they reported that both monensin, which interferes with GPCR conformational activity, and pertussis toxin, a broad-spectrum GPCR inhibitor, block shear-induced pseudopod retraction [19]. Furthermore, sheared HL60 neutrophil-like cells were shown to exhibit reduced $G_{i\alpha}$ activity in line with flow-mediated downregulation of GPCR activity. Considering fMLP stimulation above threshold concentrations blocks fluid flow-induced pseudopod retraction [44], FPR, its high affinity GPCR, was viewed as a likely candidate mechanosensor for neutrophil responses to shear.

Cell activation through fMLP stimulation, which elevates FPR activity, causes neutrophils to project pseudopods and upregulate their adhesion and migration on substrates [83]. It does so by modulating cytoskeletal remodeling, which includes an influence on the cyclical actin polymerization/depolymerization associated with pseudopod activity during cell migration [84]. F-actin dynamics are under the control of the RAS superfamily of guanine triphosphate (GTP)-binding proteins, including the small GTP-phosphatases (GTPases): Rac1, Rac2, cdc42, and the Rho GTPases [85,86]. Fluid shear stress exposure reduces Rac1 and Rac2 activity consistent with it causing retraction of existing, or preventing the formation of new, actin-enriched pseudopods by neutrophils [15]. These data further suggested a role for GPCRs as mechanotransducers of shear stress for neutrophils.

Notably, FPR appears to be critical for neutrophils to project pseudopods considering that undifferentiated HL60 cells, which do not express FPR, lack the ability to form pseudopods [19]. On the other hand, transfecting FPR expression plasmids into HL60s imparts on these cells the ability to form pseudopods that retract under the influence of fluid shear stress [19]. The key evidence implicating FPR as a shear sensor, however, was that transfecting differentiated HL-60 neutrophil-like cells with siRNA to silence FPR expression inhibited their shear-induced pseudopod retraction response, despite the fact that these cells retained the ability to project pseudopods due to the presence of other cytokine-related GPCRs [19].

While precisely how shear stress reduces FPR activity on the cell surface remains unclear, it does not involve the activity of matrix metalloproteinases (MMPs) [20], which are capable of cleaving FPR [87]. Instead, it possibly involves shear-induced internalization and relocalization of FPR from the cell surface to a perinuclear compartment [88]. Fluid shear-induced internalization of FPRs may therefore serve to minimize their constitutive activity on the cell surface, which in turn minimizes pseudopod activity. But, apparently, some FPRs must be present on the cell surface, since proteolytic FPR cleavage impairs the ability of shear stress to induce neutrophil pseudopod retraction [87].

3.2. CD18 integrins

CD18 integrins play an important role in neutrophil adhesion and migration. They form the β_2-subunit of four integrin heterodimeric complexes: lymphocyte function antigen-1 (LFA-1 or CD11a/CD18), macrophage-1 antigen (Mac-1 or CD11b/CD18), integrin $\alpha_X\beta_2$ (CD11c/CD18), and integrin $\alpha_D\beta_2$ (CD11d/CD18) [89]. Among these, CD11a/CD18 and CD11b/CD18 are critical for neutrophil-substrate interactions [18,44,90]. While CD11a/CD18 is involved in loose capture interactions that promote neutrophil arrest, CD11b/CD18 enables the neutrophil to become firmly adhered to substrates while withstanding the shearing forces applied by the surrounding blood flow. Once adhered, neutrophils become capable of migration and extravasation along chemotactic gradients toward sites of damaged tissue [1]. As a result, these two integrins play key roles in neutrophil recruitment during inflammation [91,92].

Similar to FPR, shear stress exposure downregulates expression of CD18 integrins on the neutrophil surface. This downregulation differs from FPR, however, since it relies on the enzymatic activity of proteases that cleave CD18 integrins, particularly CD11b/CD18 for neutrophils [18]. Reportedly, the CD11b and CD18 integrin subunits are cleaved by the cysteine protease cathepsin B (catB) [37,44]. Additionally, cleavage of these integrins occurs in the ectodomain presented on the outer surface of the cells [17], suggesting that catB is released from lysosomal stores into the extracellular milieu. The exact mechanisms that cause catB to be released into the extracellular milieu in response to shear stress exposure, however, remain to be elucidated.

Interestingly, CD18 integrins may act as mechanosensors. In addition to their cleavage by catB, evidence suggests that fluid shear stress exposure promotes conformational shifts in the CD18 extracellular domain [17]. These shear-induced conformational shifts appear to facilitate catB access to its cleavage site. But, it is also possible that they promote outside-in signaling and alter cell activity. Up to this point, however, the existing evidence only suggests that shear-induced CD18 cleavage removes the extracellular ligand-binding domain, thereby preventing neutrophils from adhering to substrates. As a result, this shear-induced CD11b/CD18 cleavage may not be viewed as

following the classical paradigm of mechanoreception/mechanotransduction. However, a case can be made that this shear response may represent another way by which neutrophils sense their flow environment and alter their function. In this case, CD18 integrins sense and transduce an applied shear stress stimulus into a biological event by changing their conformational activity (i.e., mechanotransduction). In turn, this mechanoreceptive event, rather than inducing cellular signaling, enables proteases to cleave integrins off the membrane surface, which manifests as a change in cell adhesion involved in a number of important neutrophil functions (e.g., phagocytosis, migration, etc.).

In summary, both FPR and CD18 integrins play crucial roles in the adhesion, migration, and defensive capabilities of human neutrophils. Furthermore, shear exposure minimizes the activity of these two surface receptors to prevent neutrophil activation under physiologic (i.e., non-inflamed) conditions. Although shear stress results in downregulation of both FPR and CD18 activity, the exact mechanotransduction mechanism differs in both cases.

4. Neutrophil Mechanosensitivity

In general, fluid shear stress mechanotransduction by vascular cells under physiological (non-diseased, non-pathogenic) conditions serves to maintain their quiescence (i.e., baseline activity) [93]. This view has evolved from a plethora of investigations regarding the various mechanosignaling pathways and mechanosensors that were conducted in a reductionist fashion where cells were analyzed under uniform and controlled stress regimes [94,95]. However, vascular cells, including the neutrophils, experience large variations in fluid stress levels from moment to moment, and they function under a diversity of mechanical stress distributions and magnitudes [96,97]. Thus, atypical stress distributions that arise due to some physiological or pathological event are unlikely to be the sole cause of chronic cell dysfunction. What may change and result in their abnormal cell activity, however, is their responsiveness to applied stresses (i.e., their mechanosensitivity).

Just as sustained chemical perturbations in the blood or changes in cell surface biochemistry may alter vascular cell activity and lead to potential pathobiology or altered tissue function, changes in cell mechanosensitivity may also impact vascular health. Along this line, the peripheral membrane appears to be in a position to serve as the mechanosensory compartment that enables cells to sense their mechanoenvironment and adjust their activity [50,98,99]. Considering that fluid flow mechanotransduction is predicated on the ability of shear stress to induce changes in the conformational activity of proteins leading to downstream signaling [100,101], it is conceivable that the mechanosensitivity of a cell depends on a number of aspects of its mechanotransducing machinery. Principally, it depends on the number of mechanosensors on the cell surface. It also may depend on the ability of these mechanosensors to deform under applied shear stresses, which likely are functions of the mechanical properties of the mechanosensor or the mechanical properties of the support medium in which the mechanosensors reside.

For neutrophils, the numbers of FPR and CD18 integrins on the cell surface influence their shear stress sensitivity. Notably, cleavage of FPR from the surfaces of neutrophils treated with proteases results in their impaired pseudopod retraction response to shear [87]. In the case of CD18 integrins, although they are cleaved in response to shear, a baseline level of their presence on the cell surface may be required to allow these cells to retract pseudopods in response to fluid shear stress. In support of this, pretreatment of neutrophils with CD18 antibodies blocks their ability to retract pseudopods under shear stress [102].

Additionally, neutrophil mechanosensitivity likely depends on the ability of mechanical stresses to alter the conformation of putative mechanosensors. Currently, there is evidence that shear stress promotes changes in the activity of many protein receptors for a number of cells, including the neutrophils. But there is little direct evidence that changes in the "deformability" of these mechanosensors will alter cell mechanosensitivity. Conceivably, this may occur as a result of genetic mutations in the amino acid sequence. Studies focusing on the inside-out related conformational shifts in integrins, which are required to promote counter-receptor binding, have demonstrated the effects of such genetic mutations. For example, point mutations to the I-domain of CD11b integrins stabilize the "open" (active) conformation of these receptors and promote ligand binding [103]. Specific point mutations to the amino acid sequence of glycoprotein IIb/IIIa also enhance ligand binding [104]. In this regard, mutations in FPR and/or CD18 integrins may alter the ability of shear stress to induce conformational activities in these receptors with an impact on neutrophil mechanosensitivity to flow. But, to date, the existence of naturally occurring genetic changes in protein structure that alter cellular responses to mechanical stimuli remains to be seen. Moreover, it is unknown whether post-translational modifications may be another source of altered mechanosensitivity.

Finally, it is possible that the physical properties of the support medium for the mechanosensors (i.e., the cell membrane) influence mechanosensitivity by affecting the ability of putative mechanoreceptors to "deform" under flow stimulation. The neutrophil membrane is the support medium for, and governs the conformational activity of, FPR and CD18 integrins. Conceivably, this includes influencing the ability of shear stress to induce conformational changes. Such a possibility is consistent with the influence of the cell membrane properties, particularly membrane fluidity, on chemokine receptor surface concentrations and affinities for ligands in the regulation of neutrophil adhesion and chemotaxis [51–54]. In the most general sense, changes in membrane fluidity likely alter receptor dynamics by influencing their intermolecular interactions (with other signaling molecules) or by modulating their structural activity due to ligand binding that controls downstream cell signaling [105]. For example, altered lipid bilayer fluidity changes the number, affinity, lateral mobility, and conformational activity of membrane receptors (e.g., GPCRs, concanavalin A receptor) [63,106]. In this fashion, membrane fluidity regulates neutrophil sensitivity to cytokine ligands that stimulate cell functions (e.g., migration, phagocytosis, growth, and differentiation) [53,54].

It turns out that membrane fluidity may also influence neutrophil mechanosensitivity. Along this line, we addressed, in a recent paper [36], the question of how changes in membrane cholesterol content associated with disease states (i.e., hypercholesterolemia) might negatively impact the anti-inflammatory control of neutrophils by fluid shear stress. Among its many biological functions, cholesterol, a structural component of the lipid bilayer, regulates the cell membrane fluidity [107]. In the context of cellular physiology, while membrane cholesterol enrichment decreases lipid bilayer fluidity, membrane cholesterol depletion increases it [108,109]. Furthermore, cholesterol-related modification of the cell membrane has been shown to alter chemokine-related signaling in neutrophils with an effect on their membrane ruffling, cell polarization, and F-actin polymerization during activation with fMLP [110,111].

Cholesterol also appears to govern cell responses to shear stresses. For example, shear-dependent activation of extracellular signal-related kinase (ERK) in endothelial cells is inhibited by depleting membrane cholesterol content [112]. Similarly, membrane cholesterol depletion alters

osteoblast mechanosignaling in response to shear stress exposure [55]. These results suggested that membrane cholesterol levels influence mechanotransduction processes, but it was unclear whether this influence is due to the effects of cholesterol on membrane fluidity or compartmentalization into lipid raft-enriched regions. Recently, we reported evidence that neutrophil mechanosensitivity depends on cholesterol-related membrane fluidity [36]. We also revealed the existence of an optimal membrane cholesterol/fluidity range that is permissive for the cell-deactivating effects of shear stress; above or below this range, the fluid shear stress responses of neutrophils are blocked [36]. Thus, the cholesterol-related fluidity of the cell membrane may be a modulator of neutrophil mechanosensitivity.

Overall, neutrophil mechanosensitivity appears to be a function not only of the mechanosensors that permit the cell to sense their local mechanoenvironment, but also of the mechanical properties of the cells themselves. In this regard, it is clear that cell mechanosensitivity may be an important parameter that determines the difference between physiology and pathobiology.

5. Neutrophil Mechanosensitivity Perspective to Cardiovascular Pathophysiology

Notably, hypertension and hypercholesterolemia are associated with chronically inflamed blood typified by sustained elevations in the numbers of activated neutrophils in the circulation [113,114]. These disease states may serve to promote microvascular inflammation and impaired tissue blood flow regulation long before the onset of lethal vascular diseases (Figure 1). This inflammation is believed to drive microcirculatory dysfunction that interferes with tissue blood flow regulation and ultimately leads to detrimental effects on the macrocirculation [1,3]. What is missing from this explanation, however, is the mitigating event that links hypertension or hypercholesterolemia with the onset of dysregulated neutrophil activity/adhesion in the microcirculation.

To date, we have accumulated evidence implicating neutrophil mechanoregulation as an important control mechanism [35]. More specifically, shear stress mechanosensitivity appears to ensure that neutrophils remain in an inactivated, quiescent state to minimize inflammatory activity in the blood in the absence of some mitigating pathogenic event. In the event that these mechanotransduction processes become dysregulated, it is conceivable that the ensuing spontaneous activation of neutrophils would promote microvascular dysfunction and, eventually, the progression of cardiovascular disease.

Recent studies suggest that dysregulated neutrophil activity may contribute to the progression of various lethal cardiovascular disease states [2,36,115,116]. There is evidence that this neutrophil dysregulation may arise, at least in some cases, due to changes in the ability of fluid shear stress to deactivate neutrophils [116]. Notably, such changes may be best described by modifications to the surface biochemistry or properties consistent with a change in their mechanosensitivity. For example, evidence has been reported that indicates the impaired shear responses of neutrophils from spontaneously hypertensive rats may be due to increased proteolytic activity in the blood that cleaves FPR receptors off the cell surface [87]. Due to FPR cleavage, pseudopods would remain extended on neutrophils, a feature linked to elevated hemodynamic resistance [115]. Neutrophils from hypercholesterolemic mice also exhibit an impaired pseudopod retraction response to fluid shear stress, which had been attributed to a potential effect of cholesterol on cell membrane fluidity [36]. These studies suggest that neutrophil mechanosensitivity is a determinant of blood health, since impaired perfusion due to pseudopod extensions may lead to tissue damage and progression of

cardiovascular diseases.

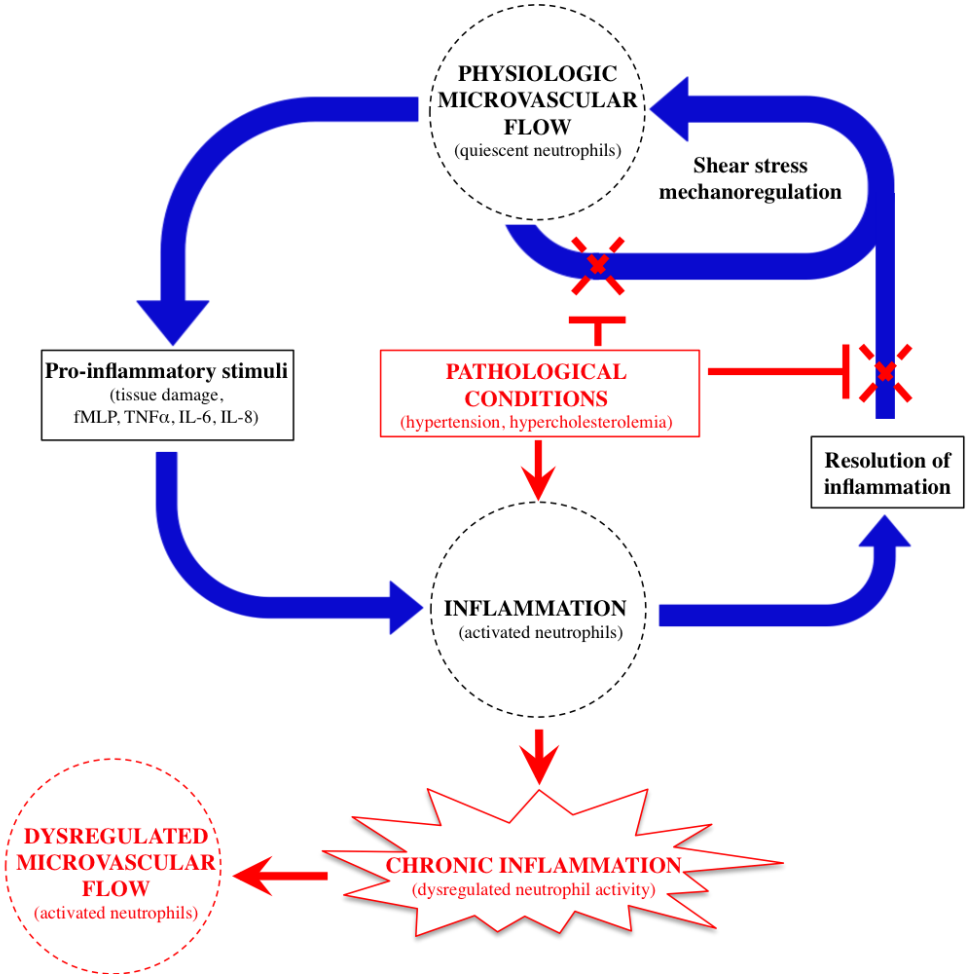

Figure 1. Proposed impact of an impaired neutrophil mechanosensitivity to shear stress on the microcirculation. Blue arrows represent the regulation of neutrophil activity under physiological (i.e., non-diseased, non-inflamed) conditions. In absence of inflammatory stimuli, neutrophils remain quiescent due, in part, to the deactivating effects of shear stress mechanoregulation. Exposure to pro-inflammatory stimuli activates neutrophils and promotes inflammation. Neutrophils remain activated until agonist levels subside during resolution of inflammation, at which point neutrophils become quiescent. The red arrows represent the effects of pathological conditions, such as hypertension or hypercholesterolemia. By impairing the neutrophil response to shear stress, these pathological conditions likely promote sustained neutrophil activation in the blood. This, in conjunction with elevated plasma levels of cytokines/agonists, eventually results in chronic inflammation. The accumulation of activated neutrophils in the microvasculature during chronic inflammation is then thought to drive dysregulated microvascular flow and downstream cardiovascular diseases.

6. Final Remarks

Recognition of shear stress mechanosensitivity as a metric of the health status of neutrophil mechanoregulation opens the door to new possibilities in terms of understanding disease pathogenesis and progression. At the same time, it provides the basis for developing novel therapeutic and diagnostic approaches to address the origins or causes of cardiovascular disease and possibly even other pathological conditions. In the end, a research strategy that adopts a global cell mechanosensitivity perspective in conjunction with a focused reductionist approach may lead to a better understanding of the contribution of mechanobiology to physiology and pathobiology.

Conflict of Interest

All authors declare no conflicts of interest in this review.

References

1. Schmid-Schonbein GW (2006) Analysis of inflammation. *Annu Rev Biomed Eng* 8: 93–131.
2. Segel GB, Halterman MW, Lichtman MA (2011) The paradox of the neutrophil's role in tissue injury. *J Leukoc Biol* 89: 359–372.
3. Mazzoni MC, Schmid-Schonbein GW (1996) Mechanisms and consequences of cell activation in the microcirculation. *Cardiovascular Research* 32: 709–719.
4. Harris AG, Skalak TC (1993) Effects of leukocyte activation on capillary hemodynamics in skeletal muscle. *Am J Physiol* 264: H909–916.
5. Helmke BP, Sugihara-Seki M, Skalak R, et al. (1998) A mechanism for erythrocyte-mediated elevation of apparent viscosity by leukocytes in vivo without adhesion to the endothelium. *Biorheology* 35: 437–448.
6. Aggarwal BB, Shishodia S, Sandur SK, et al. (2006) Inflammation and cancer: how hot is the link? *Biochem Pharmacol* 72: 1605–1621.
7. Akter K, Lanza EA, Martin SA, et al. (2011) Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? *Br J Clin Pharmacol* 71: 365–376.
8. Kim YM, Yamazaki I, Piette LH (1994) The effect of hemoglobin, hematin, and iron on neutrophil inactivation in superoxide generating systems. *Arch Biochem Biophys* 309: 308–314.
9. Miles K, Clarke DJ, Lu W, et al. (2009) Dying and necrotic neutrophils are anti-inflammatory secondary to the release of alpha-defensins. *J Immunol* 183: 2122–2132.
10. Noris M, Morigi M, Donadelli R, et al. (1995) Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circ Res* 76: 536–543
11. Tedgui A, Mallat Z (2001) Anti-inflammatory mechanisms in the vascular wall. *Circ Res* 88: 877–887.
12. Watanabe J, Lin JA, Narasimha AJ, et al. (2010) Novel anti-inflammatory functions for endothelial and myeloid cyclooxygenase-2 in a new mouse model of Crohn's disease. *Am J Physiol Gastrointest Liver Physiol* 298: G842–850.
13. Berk BC, Abe JI, Min W, et al. (2001) Endothelial atheroprotective and anti-inflammatory mechanisms. *Ann N Y Acad Sci* 947: 93–109.

14. Hsieh HJ, Liu CA, Huang B, et al. (2014) Shear-induced endothelial mechanotransduction: the interplay between reactive oxygen species (ROS) and nitric oxide (NO) and the pathophysiological implications. *J Biomed Sci* 21: 3.
15. Makino A, Glogauer M, Bokoch GM, et al. (2005) Control of neutrophil pseudopods by fluid shear: role of Rho family GTPases. *Am J Physiol Cell Physiol* 288: C863–871.
16. Shin HY, Zhang X, Makino A, et al. (2011) Mechanobiological Evidence for the Control of Neutrophil Activity by Fluid Shear Stress. In: *Mechanobiology Handbook*. CRC Press, 139–175.
17. Shin HY, Simon SI, Schmid-Schonbein GW (2008) Fluid shear-induced activation and cleavage of CD18 during pseudopod retraction by human neutrophils. *J Cell Physiol* 214: 528–536.
18. Zhang X, Zhan D, Shin HY (2013) Integrin subtype-dependent CD18 cleavage under shear and its influence on leukocyte-platelet binding. *J Leukoc Biol* 93: 251–258.
19. Makino A, Prossnitz ER, Bunemann M, et al. (2006) G protein-coupled receptors serve as mechanosensors for fluid shear stress in neutrophils. *Am J Physiol Cell Physiol* 290: C1633–1639.
20. Mitchell MJ, King MR (2012) Shear-induced resistance to neutrophil activation via the formyl peptide receptor. *Biophys J* 102: 1804–1814.
21. Papaioannou TG, Stefanadis C (2005) Vascular wall shear stress: basic principles and methods. *Hellenic J Cardiol* 46: 9–15.
22. Sheikh S, Rainger GE, Gale Z, et al. (2003) Exposure to fluid shear stress modulates the ability of endothelial cells to recruit neutrophils in response to tumor necrosis factor-alpha: a basis for local variations in vascular sensitivity to inflammation. *Blood* 102: 2828–2834.
23. Stepp DW, Nishikawa Y, Chilian WM (1999) Regulation of shear stress in the canine coronary microcirculation. *Circulation* 100: 1555–1561
24. Koutsiaris AG, Tachmitzi SV, Batis N, et al. (2007) Volume flow and wall shear stress quantification in the human conjunctival capillaries and post-capillary venules in vivo. *Biorheology* 44: 375–386
25. Marki A, Esko JD, Pries AR, et al. (2015) Role of the endothelial surface layer in neutrophil recruitment. *J Leukoc Biol*.
26. Dewitz TS, McIntire LV, Martin RR, et al. (1979) Enzyme release and morphological changes in leukocytes induced by mechanical trauma. *Blood Cells* 5: 499–512
27. Zhelev DV, Alteraifi AM, Chodniewicz D (2004) Controlled pseudopod extension of human neutrophils stimulated with different chemoattractants. *Biophys J* 87: 688–695.
28. Coughlin MF, Schmid-Schonbein GW (2004) Pseudopod projection and cell spreading of passive leukocytes in response to fluid shear stress. *Biophys J* 87: 2035–2042.
29. Komai Y, Schmid-Schonbein GW (2005) De-activation of neutrophils in suspension by fluid shear stress: a requirement for erythrocytes. *Ann Biomed Eng* 33: 1375–1386.
30. Fukuda S, Yasu T, Predescu DN, et al. (2000) Mechanisms for regulation of fluid shear stress response in circulating leukocytes. *Circ Res* 86: E13–18.
31. Moazzam F, DeLano FA, Zweifach BW, et al. (1997) The leukocyte response to fluid stress. *Proc Natl Acad Sci U S A* 94: 5338–5343.
32. Shive MS, Salloum ML, Anderson JM (2000) Shear stress-induced apoptosis of adherent neutrophils: a mechanism for persistence of cardiovascular device infections. *Proc Natl Acad Sci U S* 97: 6710–6715.

33. Lee D, Schultz JB, Knauf PA, et al. (2007) Mechanical shedding of L-selectin from the neutrophil surface during rolling on sialyl Lewis x under flow. *J Biol Chem* 282: 4812–4820.
34. Shive MS, Brodbeck WG, Anderson JM (2002) Activation of caspase 3 during shear stress-induced neutrophil apoptosis on biomaterials. *J Biomed Mater Res* 62: 163–168.
35. Makino A, Shin HY, Komai Y, et al. (2007) Mechanotransduction in leukocyte activation: a review. *Biorheology* 44: 221–249.
36. Zhang X, Hurng J, Rateri DL, et al. (2011) Membrane cholesterol modulates the fluid shear stress response of polymorphonuclear leukocytes via its effects on membrane fluidity. *Am J Physiol Cell Physiol* 301: C451–460.
37. Akenhead ML, Zhang X, Shin HY (2014) Characterization of the shear stress regulation of CD18 surface expression by HL60-derived neutrophil-like cells. *Biomech Model Mechanobiol* 13: 861–870.
38. Sugihara-Seki M, Schmid-Schonbein GW (2003) The fluid shear stress distribution on the membrane of leukocytes in the microcirculation. *J Biomech Eng* 125: 628–638
39. Conway DE, Breckenridge MT, Hinde E, et al. (2013) Fluid shear stress on endothelial cells modulates mechanical tension across VE-cadherin and PECAM-1. *Curr Biol* 23: 1024–1030.
40. Goldmann WH (2012) Mechanotransduction and focal adhesions. *Cell Biol Int* 36: 649–652.
41. Kamm RD, Kaazempur-Mofrad MR (2004) On the molecular basis for mechanotransduction. *Mech Chem Biosyst* 1: 201–209
42. Lee SE, Kamm RD, Mofrad MR (2007) Force-induced activation of talin and its possible role in focal adhesion mechanotransduction. *J Biomech* 40: 2096–2106.
43. Jannat RA, Robbins GP, Ricart BG, et al. (2010) Neutrophil adhesion and chemotaxis depend on substrate mechanics. *J Phys Condens Matter* 22: 194117.
44. Fukuda S, Schmid-Schonbein GW (2003) Regulation of CD18 expression on neutrophils in response to fluid shear stress. *Proc Natl Acad Sci U S A* 100: 13152–13157.
45. Su SS, Schmid-Schonbein GW (2008) Fluid stresses on the membrane of migrating leukocytes. *Ann Biomed Eng* 36: 298–307.
46. Seely AJ, Pascual JL, Christou NV (2003) Science review: Cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance. *Crit Care* 7: 291–307.
47. Tarbell JM, Pahakis MY (2006) Mechanotransduction and the glycocalyx. *J Intern Med* 259: 339–350.
48. White CR, Frangos JA (2007) The shear stress of it all: the cell membrane and mechanochemical transduction. *Philos Trans R Soc Lond B Biol Sci* 362: 1459–1467.
49. Bodin S, Welch MD (2005) Plasma membrane organization is essential for balancing competing pseudopod- and uropod-promoting signals during neutrophil polarization and migration. *Mol Biol Cell* 16: 5773–5783.
50. Butler PJ, Norwich G, Weinbaum S, et al. (2001) Shear stress induces a time- and position-dependent increase in endothelial cell membrane fluidity. *Am J Physiolp Cell Physiolp* 280: C962–969.
51. Berlin RD, Fera JP (1977) Changes in membrane microviscosity associated with phagocytosis: effects of colchicine. *Proc Natl Acad Sci U S A* 74: 1072–1076.
52. Wiles ME, Dykens JA, Wright CD (1994) Regulation of polymorphonuclear leukocyte membrane fluidity: effect of cytoskeletal modification. *Journal of leukocyte biology* 56: 192–199.

53. Tomonaga A, Hirota M, Snyderman R (1983) Effect of membrane fluidizers on the number and affinity of chemotactic factor receptors on human polymorphonuclear leukocytes. *Microbiol Immunol* 27: 961–972.
54. Yuli I, Tomonaga A, Synderman R (1982) Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers. *Proc Natl Acad Sci U S A* 79: 5906–5910.
55. Ferraro JT, Daneshmand M, Bizios R, et al. (2004) Depletion of plasma membrane cholesterol dampens hydrostatic pressure and shear stress-induced mechanotransduction pathways in osteoblast cultures. *Am J Physiol Cell Physiol* 286: C831–839.
56. Rizzo V, McIntosh DP, Oh P, et al. (1998) In situ flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. *J Biol Chem* 273: 34724–34729.
57. Rizzo V, Sung A, Oh P, et al. (1998) Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. *J Biol Chem* 273: 26323–26329.
58. Niggli V, Meszaros AV, Oppliger C, et al. (2004) Impact of cholesterol depletion on shape changes, actin reorganization, and signal transduction in neutrophil-like HL-60 cells. *Exp Cell Res* 296: 358–368.
59. Tuluc F, Meshki J, Kunapuli SP (2003) Membrane lipid microdomains differentially regulate intracellular signaling events in human neutrophils. *Int Immunopharmacol* 3: 1775–1790.
60. Marwali MR, Rey-Ladino J, Dreolini L, et al. (2003) Membrane cholesterol regulates LFA-1 function and lipid raft heterogeneity. *Blood* 102: 215–222.
61. Solomkin JS, Robinson CT, Cave CM, et al. (2007) Alterations in membrane cholesterol cause mobilization of lipid rafts from specific granules and prime human neutrophils for enhanced adherence-dependent oxidant production. *Shock* 28: 334–338.
62. Cattaruzza M, Dimigen C, Ehrenreich H, et al. (2000) Stretch-induced endothelin B receptor-mediated apoptosis in vascular smooth muscle cells. *FASEB J* 14: 991–998.
63. Chachisvilis M, Zhang YL, Frangos JA (2006) G protein-coupled receptors sense fluid shear stress in endothelial cells. *Proc Natl Acad Sci U S A* 103: 15463–15468.
64. Yasuda N, Miura S, Akazawa H, et al. (2008) Conformational switch of angiotensin II type 1 receptor underlying mechanical stress-induced activation. *EMBO Rep* 9: 179–186.
65. Zhang YL, Frangos JA, Chachisvilis M (2009) Mechanical stimulus alters conformation of type 1 parathyroid hormone receptor in bone cells. *Am J Physiol Cell Physiol* 296: C1391–1399.
66. Zou Y, Akazawa H, Qin Y, et al. (2004) Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol* 6: 499–506.
67. Chen KD, Li YS, Kim M, et al. (1999) Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc. *J Biol Chem* 274: 18393–18400.
68. Iwasaki H, Eguchi S, Ueno H, et al. (2000) Mechanical stretch stimulates growth of vascular smooth muscle cells via epidermal growth factor receptor. *Am J Physiol Heart Circ Physiol* 278: H521–529.
69. Jin ZG, Ueba H, Tanimoto T, et al. (2003) Ligand-independent activation of vascular endothelial growth factor receptor 2 by fluid shear stress regulates activation of endothelial nitric oxide synthase. *Circ Res* 93: 354–363.
70. Lee HJ, Koh GY (2003) Shear stress activates Tie2 receptor tyrosine kinase in human endothelial cells. *Biochem Biophys Res Commun* 304: 399–404.

71. Milkiewicz M, Doyle JL, Fudalewski T, et al. (2007) HIF-1alpha and HIF-2alpha play a central role in stretch-induced but not shear-stress-induced angiogenesis in rat skeletal muscle. *J Physiol* 583: 753–766.
72. Palumbo R, Gaetano C, Melillo G, et al. (2000) Shear stress downregulation of platelet-derived growth factor receptor-beta and matrix metalloprotease-2 is associated with inhibition of smooth muscle cell invasion and migration. *Circulation* 102: 225–230.
73. Shay-Salit A, Shushy M, Wolfovitz E, et al. (2002) VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells. *Proc Natl Acad Sci U S A* 99: 9462–9467.
74. Gu CX, Juranka PF, Morris CE (2001) Stretch-activation and stretch-inactivation of Shaker-IR, a voltage-gated K+ channel. *Biophys J* 80: 2678–2693.
75. Maingret F, Patel AJ, Lesage F, et al. (1999) Mechano- or acid stimulation, two interactive modes of activation of the TREK-1 potassium channel. *J Biol Chem* 274: 26691–26696
76. Tarbell JM, Weinbaum S, Kamm RD (2005) Cellular fluid mechanics and mechanotransduction. *Ann Biomed Eng* 33: 1719–1723.
77. Haugh MG, Vaughan TJ, McNamara LM (2015) The role of integrin alpha(V)beta(3) in osteocyte mechanotransduction. *J Mech Behav Biomed Mater* 42: 67–75.
78. Teravainen TP, Myllymaki SM, Friedrichs J, et al. (2013) alphaV-integrins are required for mechanotransduction in MDCK epithelial cells. *PloS one* 8: e71485.
79. Watabe H, Furuhama T, Tani-Ishii N, et al. (2011) Mechanotransduction activates alpha(5)beta(1) integrin and PI3K/Akt signaling pathways in mandibular osteoblasts. *Exp Cell Res* 317: 2642–2649.
80. Gudi S, Nolan JP, Frangos JA (1998) Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition. *Proc Natl Acad Sci U S A* 95: 2515–2519.
81. Gudi SR, Clark CB, Frangos JA (1996) Fluid flow rapidly activates G proteins in human endothelial cells. Involvement of G proteins in mechanochemical signal transduction. *Circ Res* 79: 834–839.
82. Migeotte I, Communi D, Parmentier M (2006) Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev* 17: 501–519.
83. Gerisch G, Keller HU (1981) Chemotactic reorientation of granulocytes stimulated with micropipettes containing fMet-Leu-Phe. *J Cell Sci* 52: 1–10.
84. Welch MD, Mallavarapu A, Rosenblatt J, et al. (1997) Actin dynamics in vivo. *Curr Opin Cell Biol* 9: 54–61.
85. Hall A (1994) Small GTP-binding proteins and the regulation of the actin cytoskeleton. *Ann Rev Cell Biol* 10: 31–54.
86. Ridley AJ (2001) Rho family proteins: coordinating cell responses. *Trends Cell Biol* 11: 471–477.
87. Chen AY, DeLano FA, Valdez SR, et al. (2010) Receptor cleavage reduces the fluid shear response in neutrophils of the spontaneously hypertensive rat. *Am J Physiol Cell Physiol* 299: C1441–1449.
88. Su SS, Schmid-Schonbein GW (2010) Internalization of Formyl Peptide Receptor in Leukocytes Subject to Fluid Stresses. *Cell Mol Bioeng* 3: 20–29.
89. Mazzone A, Ricevuti G (1995) Leukocyte CD11/CD18 integrins: biological and clinical relevance. *Haematologica* 80: 161–175.

90. Root RK (1990) Leukocyte adhesion proteins: their role in neutrophil function. *Trans Am Clin Climatol Assoc* 101: 207-224; discussion 224–206.
91. Diacovo TG, Roth SJ, Buccola JM, et al. (1996) Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. *Blood* 88: 146–157.
92. Hentzen ER, Neelamegham S, Kansas GS, et al. (2000) Sequential binding of CD11a/CD18 and CD11b/CD18 defines neutrophil capture and stable adhesion to intercellular adhesion molecule-1. *Blood* 95: 911–920.
93. Paszkowiak JJ, Dardik A (2003) Arterial wall shear stress: observations from the bench to the bedside. *Vascular Endovascular Surgery* 37: 47–57.
94. Shyy JY, Chien S (2002) Role of integrins in endothelial mechanosensing of shear stress. *Circ Res* 91: 769–775.
95. Tzima E, Irani-Tehrani M, Kiosses WB, et al. (2005) A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* 437: 426–431.
96. Lipowsky HH (2005) Microvascular rheology and hemodynamics. *Microcirculation* 12: 5–15.
97. Pohlman TH, Harlan JM (2000) Adaptive responses of the endothelium to stress. *J Surg Res* 89: 85–119.
98. Butler PJ, Tsou TC, Li JY, et al. (2002) 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* 16: 216–218.
99. Haidekker MA, L'Heureux N, Frangos JA (2000) Fluid shear stress increases membrane fluidity in endothelial cells: a study with DCVJ fluorescence. *Am J Physiol Heart Circ Physiol* 278: H1401–1406.
100. Friedland JC, Lee MH, Boettiger D (2009) Mechanically activated integrin switch controls alpha5beta1 function. *Science* 323: 642–644.
101. Puklin-Faucher E, Gao M, Schulten K, et al. (2006) How the headpiece hinge angle is opened: New insights into the dynamics of integrin activation. *J Cell Biol* 175: 349–360.
102. Marschel P, Schmid-Schonbein GW (2002) Control of fluid shear response in circulating leukocytes by integrins. *Ann Biomed Eng* 30: 333–343.
103. McCleverty CJ, Liddington RC (2003) Engineered allosteric mutants of the integrin alphaMbeta2 I domain: structural and functional studies. *Biochem J* 372: 121–127.
104. Luo BH, Karanicolas J, Harmacek LD, et al. (2009) Rationally designed integrin beta3 mutants stabilized in the high affinity conformation. *J Biol Chem* 284: 3917–3924.
105. Lenaz G (1987) Lipid fluidity and membrane protein dynamics. *Biosci Rep* 7: 823–837.
106. Ben-Bassat H, Polliak A, Rosenbaum SM, et al. (1977) Fluidity of membrane lipids and lateral mobility of concanavalin A receptors in the cell surface of normal lymphocytes and lymphocytes from patients with malignant lymphomas and leukemias. *Cancer Res* 37: 1307–1312.
107. Yeagle PL (1991) Modulation of membrane function by cholesterol. *Biochimie* 73: 1303–1310.
108. Chabanel A, Flamm M, Sung KL, et al. (1983) Influence of cholesterol content on red cell membrane viscoelasticity and fluidity. *Biophys J* 44: 171–176.
109. Cooper RA (1978) Influence of increased membrane cholesterol on membrane fluidity and cell function in human red blood cells. *J Supramol Struct* 8: 413–430.
110. Khatibzadeh N, Spector AA, Brownell WE, et al. (2013) Effects of plasma membrane cholesterol level and cytoskeleton F-actin on cell protrusion mechanics. *PloS one* 8: e57147.

111.Pierini LM, Eddy RJ, Fuortes M, et al. (2003) Membrane lipid organization is critical for human neutrophil polarization. *J Biol Chem* 278: 10831–10841.
112.Park H, Go YM, St John PL, et al. (1998) Plasma membrane cholesterol is a key molecule in shear stress-dependent activation of extracellular signal-regulated kinase. *J Biol Chem* 273: 32304–32311.
113.Shen K, DeLano FA, Zweifach BW, et al. (1995) Circulating leukocyte counts, activation, and degranulation in Dahl hypertensive rats. *Circ Res* 76: 276–283.
114.Tatsukawa Y, Hsu WL, Yamada M, et al. (2008) White blood cell count, especially neutrophil count, as a predictor of hypertension in a Japanese population. *Hypertens Res* 31: 1391–1397.
115.Fukuda S, Yasu T, Kobayashi N, et al. (2004) Contribution of fluid shear response in leukocytes to hemodynamic resistance in the spontaneously hypertensive rat. *Circ Res* 95: 100–108.
116.Zhang X, Cheng R, Rowe D, et al. (2014) Shear-sensitive regulation of neutrophil flow behavior and its potential impact on microvascular blood flow dysregulation in hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 34: 587–593.

AIMS Press