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Mechanotransduction in neutrophil activation and deactivation☆

Andrew E. Ekpenyong [a,b], Nicole Toepfner [b,c], Edwin R. Chilvers [d], Jochen Guck [b,*]

[a] Department of Physics, Creighton University, Omaha, NE 68178, USA
[b] Biotechnology Center, Technische Universität Dresden, Dresden, Germany
[c] Klinik und Poliklinik für Kinder- und Jugendmedizin, Universitätsklinikum Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[d] Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's and Papworth Hospitals, Cambridge CB2 0QQ, UK

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ABSTRACT

Mechanotransduction refers to the processes through which cells sense mechanical stimuli by converting them to biochemical signals and, thus, eliciting specific cellular responses. Cells sense mechanical stimuli from their 3D environment, including the extracellular matrix, neighboring cells and other mechanical forces. Incidentally, the emerging concept of mechanical homeostasis,long term or chronic regulation of mechanical properties, seems to apply to neutrophils in a peculiar manner, owing to neutrophils' ability to dynamically switch between the activated/primed and deactivated/deprimed states. While neutrophil activation has been known for over a century, its deactivation is a relatively recent discovery. Even more intriguing is the reversibility of neutrophil activation and deactivation. We review and critically evaluate recent findings that suggest physiological roles for neutrophil activation and deactivation and discuss possible mechanisms by which mechanical stimuli can drive the oscillation of neutrophils between the activated and resting states. We highlight several molecules that have been identified in neutrophil mechanotransduction, including cell adhesion and transmembrane receptors, cytoskeletal and ion channel molecules. The physiological and pathophysiological implications of such mechanically induced signal transduction in neutrophils are highlighted as a basis for future work. This article is part of a Special Issue entitled: Mechanobiology.

1. Introduction

Neutrophils are white blood cells that have banded or segmented nuclei with 3 to 5 lobes [1,2] (see Fig. 1). Thus, they are also called polymorphonuclear (PMN) leukocytes [3,4]. With over 200 cell types in the human body [5], all sharing basically the same genome, it is remarkable that neutrophils can be unambiguously identified based on a physical property, namely, nuclear morphology, visible via light microscopy, sometimes without staining and without separation medium [6–9]. In fact, nuclear morphology is used in clinical settings as I/T ratio, distinguishing immature (I) PMNs from the total neutrophil count (T) [10,11]. Neutrophils can also be identified based on specific surface markers such as CD66a [12] and CD66b [12,13]. The nuclei of mature neutrophils stay multi-lobulated and remain intact [1], as neutrophils emerge from the bone marrow where they are born/generated/constituted, into circulating blood and into tissues where they undertake anti-microbial, pro-inflammatory and other functions. The tenacity of this physical attribute (nuclear lobulation) makes it possible to monitor them throughout their lifespan. Interestingly, their lifespan at different stages involves very different 3D environments, contact with different cell types and exposure to a broad range of biochemical and mechanical stimuli. These stimuli enable neutrophils to switch from a quiescent or resting state, with a completely round shape, to a primed or activated state with an amoeboid morphology (Fig. 1).

Priming can be described as a process that gives to neutrophils in the resting state a functional response that can become greatly amplified upon exposure to another stimulus [15,16]. The second stimulus is usually regarded as the activating agent or agonist. The amplified functional response leaves neutrophils in an activated state. Thus, full neutrophil activation is a two-step process, beginning with priming by an initial exposure to priming agents such as cytokines (e.g., GM-CSF, IL-1α, TNF-α) and antigens (e.g. pathogen endotoxins). Priming and activation clearly take neutrophils from a so-called resting state to an activated state (Fig. 1), enabling them to perform their antibacterial, pro-and anti-inflammatory functions.

There are several reviews and research highlights dealing with the life of neutrophils from their differentiation and release from the bone marrow (into the blood) to their death and clearance [2,15–27]. Although they overlap in the basics, the reviews focus on some aspects worthy of mention. There are detailed presentations of the roles of neutrophils not just as the most abundant immune cells in the human body, which kill bacteria, promote tissue inflammation and repair, but also as cells which can inform and shape acquired or adaptive immune

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* Corresponding author.
E-mail address: jochen.guck@tu-dresden.de (J. Guck).

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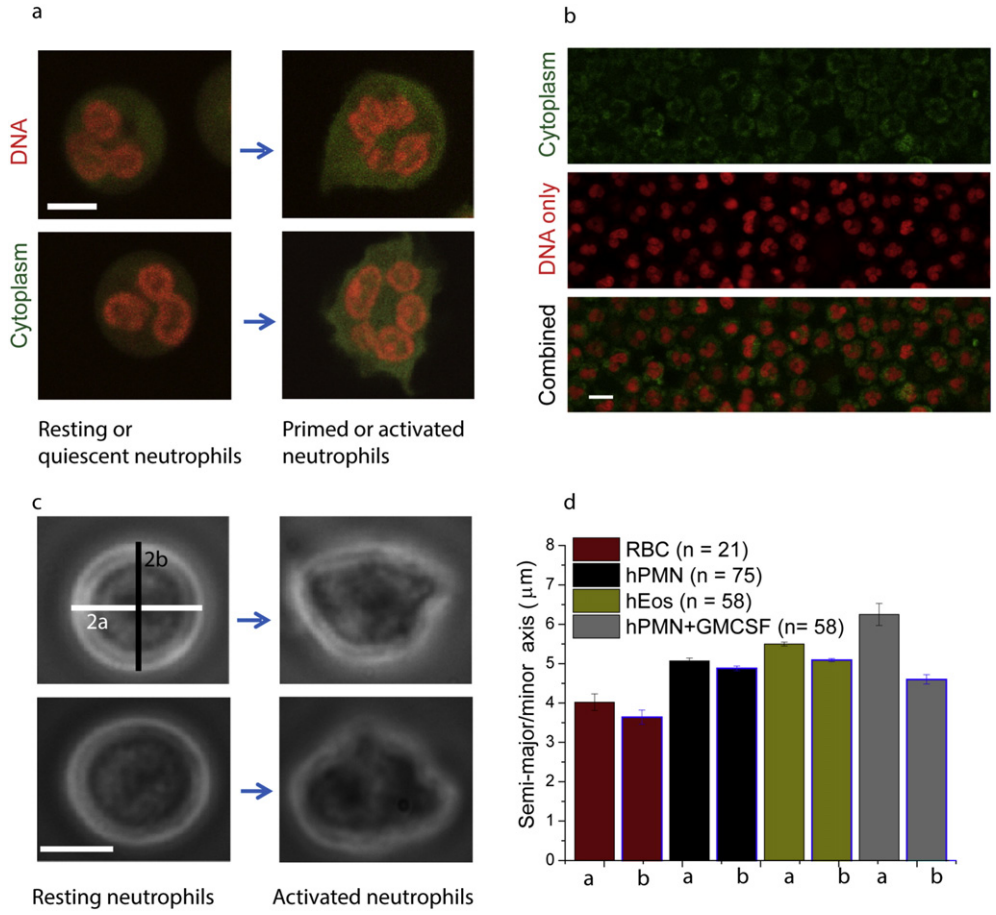

Fig. 1. Neutrophil morphological changes in response to chemical and mechanical stimuli [14]. (a) Confocal images of Syto 61 (red nuclear dye) and mitotracker orange- (green cytoplasmic dye) stained neutrophils. The resting cells remain round while activated cells become amoeboid in shape. (b) Confocal images of stained neutrophils as in (a) with separate and combined channels. Nuclei of neutrophils have 3–5 lobes, making it easy to identify them. (c) Phase contrast images of resting and activated neutrophils used in morphometry where the semimajor and minor axes (a, b) are indicated. (d) Morphometry of resting neutrophils compared with red blood cells (RBCs), eosinophils (hEos) and activated neutrophils (hPMN + GMCSF). The scale bars are 5 μm in (a) and (c) and 10 μm in (b).

responses [23,24]. There is a recent discussion on the possible existence of different subsets of neutrophils [26] as well as a new emphasis on differences between neutrophils in various functional compartments [28]. There are expositions of the function and malfunction of neutrophils in several diseases [15,21], in inflammatory diseases [22,27], infectious diseases [2,20] and in cardiovascular diseases [27]. These reviews unequivocally point to the rapidly growing evidence for the relevance of neutrophils in disease pathogenesis and implement neutrophil (mal)-function as an important focus to improve disease diagnosis and to increase therapeutic options in clinical settings.

In general, the functions and malfunctions of neutrophils in the reviews cited above are largely those amenable to in vitro, ex vivo and in vivo biochemical assessment. Neutrophil functions that are amenable to in vitro and ex vivo biochemical assessment include calcium mobilization [29], F-actin assembly [30], adhesion [31], aggregation [13], degranulation [19], phagocytosis [21] and reactive oxygen species (ROS) production [32]. Those which can be quantified in vivo include the formation of neutrophil extracellular traps or NETs [33], recruitment and migration [34]. However, several of these neutrophil functions involve not just alterations in the biochemical properties but also modifications of the physical properties of neutrophils such as their shape [35,36], size [37], deformability [4,38] and other mechanical properties [39–41]. Now, the mechanical properties of cells in general have progressed from being inherent biophysical parameters that are promising for therapeutic considerations [42], cell characterization [43], marking malignant transformation [44], cancer diagnosis [45], to constituting candidate clinical parameters [46], albeit with challenges yet to be overcome. Such challenges include adequate and reliable methods for measuring cell mechanical properties within clinical settings. A recently published method, real-time deformability cytometry (RT-DC) [47] has addressed these challenges and widespread clinical use is envisaged. Other methods for assessing cell mechanical properties have been reviewed in view of possible translation to the clinic [46,48]. Obviously, clinical translation has given impetus to both the development of techniques for measuring cell mechanical properties and detailed studies on the physiological roles of such properties. In addition, there is a recent and growing understanding of the importance of mechanotransduction in the physiology of cells in general [49]. But what is mechanotransduction?

Mechanotransduction is a type of signal transduction initiated by mechanical stimuli. It refers to the processes by which cells sense mechanical stimuli by converting them to biochemical signals, thus, eliciting specific cellular responses [50,51]. The mechanical stimuli can arise from cell to cell interactions, from the contact between cells and the 3D extracellular matrix (ECM) or from other stress-imparting events such as normal and fluid shear stresses. There is a paucity of literature focusing on neutrophil mechanotransduction [52], in spite of the latter being part of the aforementioned biophysical modifications accompanying neutrophil functions.

Here, we present a synopsis of what is currently known about the mechanobiology of primary neutrophils during their production, functions, malfunctions, clearance and pathological accumulation. Since, PMNs undergo several physical and biochemical alterations while in different environments (bone marrow, blood, tissues) and

while performing different functions, we summarize what is known about neutrophil mechanical properties and response to mechanical stimuli (mechanotransduction) while in these environments and while performing their functions. We consider particularly those mechanosensitive changes that might be characteristic of pathology. We speculate on their diagnostic value and discuss the possibility of using them as therapeutic targets. First, we give a propaedeutic survey of PMN morphometry and time-dependent events which impinge on interpretation of neutrophil mechanosensitivity in the second section. In the third section, we trace out the contexts for the mechanical properties and sensitivity to forces of neutrophils from their production in the bone marrow (BM), to their circulation in the blood and their migration into tissues, highlighting the functional implications of such properties and sensitivity. In the fourth section, we discuss controversial rheological and morphometric results, the response of neutrophils to mechanical stimuli, the possible molecular mechanism(s) that effect such responses and current challenges relevant for neutrophil biology. Throughout these sections, we note areas where further mechanical characterization and further mechanotransduction studies might be needed and these areas are succinctly presented in the concluding remarks.

2. Neutrophil states, morphometry, timescales and mechanotransduction

2.1. Neutrophil states: resting versus activated

A detailed description of resting versus primed/activated PMNs is shown in Table 1. We tabulate the differences between resting and activated neutrophils, with emphasis on mechanics-related features.

The principal consequences of priming/activation include alterations in cell polarization and deformability, expression of integrins/selectins, generation of superoxide anions (by secretagogues-substances that cause other substances to be secreted) and the release of degranulation and lipid mediators (e.g. leukotriene B_4 and arachidonic acid) [16]. When primed/activated using chemoattractants on PMNs in suspension, such as GM-CSF or fMLP, the following biophysical changes have been documented in addition to cell polarization: rapid redistribution of F-actin from a diffuse central location in the cytoplasm to a ring-like formation on the periphery of cells, and rapid reorganization of cell surface morphological features, with accumulation of plasma membrane in the front of polar cells [36]. While chemokines and cytokines can induce activation, other stimuli such as glass surfaces and exposure to biofilms can lead to similar effects. Moreover, mechanical stimuli can prime and activate neutrophils. Deformation in narrow channels led to activation and pseudopod formation [14,53], contactless stretching by optical forces yielded similar results [14].

2.2. Morphometry and response to mechanical stimuli

Neutrophils exhibit dramatic morphological changes in response to chemical or mechanical stimuli [14,53,59] (see Fig. 1). Hence, we consider neutrophil morphology in some detail. Cellular mechanical properties are endowed largely by the cytoskeleton [60]. But the cytoskeleton also gives structural integrity to cells and determines their shape [36,61]. Hence, shape, size and mechanical properties are related as readouts of cytoskeletal architecture. For a recent, detailed and statistically robust measurement of human neutrophil morphometric parameters including cell shape and size, nuclear shape and size, see [37]. Table 2 is a purview of direct measurements as well as deduced measurements of PMN morphology, and the relevant morphology of their environment. Next, we indicate the connection between these morphometric parameters and the mechanical properties, in view of their responses to mechanical stimuli.

Back in the early 1960s, and largely for anatomical reasons, Weibel [65] and Spencer [66] published separate papers on the morphometry of the human lungs, including data on the size of capillary segments. In 1980, Schönbein et al. published work on the morphometry of human leukocytes [67] using transmission electron microscopy and light microscopy. They gave hPMN size. Later (1985), Chien compared PMN and RBC deformability [68], showing that RBCs were much more deformable than PMNs. Shortly after (1987), Hogg, in a review, "Neutrophil kinetics and lung injury" [69], used the aforementioned works to hypothesize that the less deformable PMNs would have a longer transit time through the pulmonary capillaries, compared to RBCs. Both cell types would nevertheless be delayed by the capillary segments that are narrower than cell diameters, since the cells would have to be deformed in order to pass through them. These hypotheses were supported in 1993 by a group led by Hogg [4]. They measured and compared the diameters of leporine, canine and human neutrophils and capillary segments (see Table 2). They also observed the shapes of neutrophils passing through the capillaries. The human studies were carried out ex vivo on tissues obtained from four patients undergoing tumor-related lung resection while the animal studies were conducted in situ. The authors confirmed that PMNs are deformed to elliptical shapes by constrictions in pulmonary capillaries and that these constrictions slow down the transit of PMNs through the pulmonary microcirculation. With this advancement in physiological knowledge based on morphometry and mechanical properties, the next hypothesis presented itself: can neutrophils 'deficient' in deformability lead to pulmonary disorders involving inappropriate accumulation of neutrophils in the pulmonary microcirculation?

Just as clinical challenges initiated and sustained interest in neutrophil mechanical properties, other observations in the context of extracorporeal circulation led to reports about major shape changes in

Table 1
Differences between resting and primed neutrophils (human).

	Resting PMNs	Primed and activated PMNs	Source
1	Remain completely round, no pseudopods, smooth plasma membrane	Form pseudopods, are polarized with leading edge, some are round but ruffled or round but ridged	[36,53]
2	Do not spread and do not migrate	Can spread on substrate and migrate	[22]
3	Short-lived, apoptosis within 12–18 h, regulated by Mcl-1	Delayed apoptosis, secretion of pro-inflammatory cytokines and chemokines, presentation of antigens to T-cells.	[54]
4	Smaller than primed or activated cells	Larger 2D surface area than resting cells. Volume presumably larger	[36]
5	Can hardly synthesize IL-1, -1RA, -6, -12, TGF-β, TNF-α, oncostatin M and BLyS	Can synthesize IL-1, -1RA,-−6, -12, TGF-β, TNF-α, oncostatin M and BLyS	[22,55]
6	FcγRI, a high-affinity IgG receptor that binds monomeric IgG, is not expressed on resting blood neutrophils	FcγRI, is expressed on primed and activated neutrophils	[22]
7	A resting PMN has very little capacity to produce ROS, as NADPH oxidase is a multi-component enzyme that is assembled at the plasma membrane during priming	Increased ROS production is a hallmark of primed and activated PMNs	[19,32,56]
8	Local stiffness is generally more homogeneous than in primed cells	Central regions stiffer (1548 ± 871 Pa) than leading edge (686 ± 801 Pa) and tail regions (494 ± 537 Pa) measured with atomic force microscope	[57]
9	Non-macrophage-like	Can perform most if not all functions of macrophages	[22]
10	High basal intracellular chloride (Cl-i) levels	Low intracellular chloride level	[58]

Table 2
Length scales of neutrophil structure and related structures.

	Structure	Size	Source
1	Diameter of PMNs	7–11 μm	[2,62,37]
2	61%, 67%, and 38% of the pulmonary capillary segments are narrower than the mean diameter of spherical PMNs in the rabbit, dog, and human, respectively	6, 6, 7 μm respectively (average diameter of capillary segment)	[4]
3	Average length of capillaries	1 mm	[63]
4	Average width of pulmonary capillaries	2–15 μm	[4]
5	Typical diameter of neutrophil granule	300 nm	[64]

neutrophils induced by mechanical stimuli, as early as 1977 in a work aptly titled "Mechanical Trauma in Leukocytes" [59]. This work by McIntyre and his colleagues appears to be the first report of neutrophil priming/activation by mechanical stimuli and therefore the earliest work on neutrophil mechanotransduction. Apart from the authors in subsequent works, "Mechanical Trauma in Leukocytes" was not cited in the context of cell mechanics/mechanotransduction until 1997 [70]. Rather, the aforementioned interest in neutrophil morphometry and mechanical properties advanced along the lines of underlying cytoskeletal dynamism of neutrophils, bringing to the fore, timescale dependence of neutrophil morphology and mechanical properties.

2.3. Timescales

The cytoskeleton is a dynamic structure whose material properties can be quite different at short timescales compared to long timescales [61]. There is emerging evidence that such timescale dependence of cytoskeletal or cellular mechanical properties correlate with different cellular functions [71]. In the literature, the time it takes PMNs to perform certain functions (see Table 3) have sometimes been interpreted as indirect read outs of PMN mechanical properties [38,72]. Also the times required for cytoskeletal rearrangements have been used as indicators of concomitant changes in PMN mechanical properties [53,73]. Moreover, the F-actin content of the cytoskeleton has been used as a measure of PMN deformability [36,74]. As a propaedeutic section, it is important to note here that relatively long timescale functions such as migration (minutes) and short timescale functions such as advection through capillary constrictions (seconds) might indeed require different cell mechanical properties, as recently demonstrated in vitro [71], and perhaps, different cytoskeletal architectures, which is yet to be shown.

Furthermore, it becomes necessary to care about the temporal resolution of the technique of measurement as well as the time evolution of the property involved (see [38] as a clear example of time-dependent changes in PMN deformability). Time-dependent changes in the biophysical properties of PMNs are expected to be traceable to gene expression and epigenetic regulation in general. For instance, when PMNs were exposed to LPS, *Escherichia coli* or fMLP in gene expression studies, clustering analysis showed different time-dependent profiles due to many rapid changes in gene expression within the first hour of exposure [55]. However, the epigenetic details are beyond the scope of this review.

Another point that impinges upon interpretation of results is the type of mechanical property probed. Measuring the local stiffness [78] of the cytoplasm may not always correlate with whole cell rheological measurements [41]. Instead of a glossary on mechanical properties, we give a conspectus of actual mechanical parameters of PMNs measured and comment on their context, with a view to alerting the reader about the need to connect the conceptual underpinnings of such parameters with the relevant PMN physiological phenomena. This will lead us to a chronology of neutrophil mechanotransduction studies where in vivo or in vivo-like mechanical stimuli were applied to neutrophils and their responses assessed.

2.4. Neutrophil mechanical properties and mechanotransduction

In this section, we present the literature showing measurements undertaken only on primary human neutrophils (see Table 4). In the column 'technique used' of Table 4, we give relevant introductory paper(s) on the technique, while in 'source', we indicate the paper(s) reporting mechanical characterization of primary human neutrophils. The entries in Table 4 are selected as representations of clinically relevant studies: hence, studies done on single human PMNs from healthy donors and patients are included.

The mechanical parameters include measurements of transit times being presented as *deformability*[38,79], a glass-contact and aspiration based measurement of strain being denoted as *deformability*[80], as well as a contactless hydrodynamic measurement of strain still defined as *deformability*[47,81,82]. Considering the timescales involved in PMN activation (see Table 3) by mechanical perturbation [53,73] including contacts, it is important to distinguish carefully between contact-based and contactless measurements of strain. But strain alone or deformability is not enough. Biological cells in general are viscoelastic materials [83–85] and can tune their viscous and elastic properties in peculiarly time-dependent ways [38,71,76,86].

It has been noted that PMN viscoelastic properties can fluctuate between predominantly viscous to predominantly elastic responses following deformation, depending on duration of deformation [38]. Thus, measurements yielding elastic and storage moduli (see Table 4) and other elastic/viscous parameters [41,71,78,87] seem to be more clinically relevant when considering the role of cell mechanics in PMN functions and malfunctions. Clarity in rheological nomenclature [88] and an intuitive understanding of what it means for a cell to be less *rigid*, more *compliant*, more *deformable*, more *viscous*, less *elastic* (all of which have been used in the papers cited in Table 4) is necessary for accurate interpretation of experimental results on neutrophil function and for the understanding of neutrophil-mediated pathophysiology. We will return to these issues in the discussion section.

Table 3
Time scales of neutrophil functions and related events.

	Activity/function	Duration/speed	Source
1	Human PMN crossing of the endothelium (in vitro assay)	0.6–1.2 min	[39]
2	Pulmonary transit speed of murine blood cells	Over 1 mm/s	[75]
3	Gas exchange across pulmonary capillary	0.25 s	[63]
4	Time for rapid stiffening of hPMN treated with fMLP (partial recovery in 5 min following fMLP treatment)	2–3 min	[38]
5	Time for shape recovery of passive neutrophils after deformation into sausage-like shape in glass micropipette	1 min	[76]
6	Time for recovery to nearly the initial values of viscoelastic moduli due to passage through narrow channels	1 min	[53]
7	Time for rapid depolymerization and subsequent recovery of F-actin upon deformation through narrow polycarbonate filter pores	Minutes	[73]
8	Transit time of resting human neutrophils across pulmonary circulation	14.2 ± 0.3 s	[77]
9	Time to onset of neutropenia (with massive sequestration of PMNs within alveolar capillaries) due to systemic activation of neutrophils by intravenous injection of chemoattractants such as interleukin (IL)-8	Less than 1 min	[17]

Table 4
Parameters used in mechanical phenotyping of primary human neutrophils.

	Parameter and context for measurement	Unit	Technique used	Source
1	Non-contact compliance of the whole hPMN (from venous blood). Elastic and viscous parameters extractable.	Pa^{-1}	Optical stretcher [89,90]	[41,71]
2	Elastic and viscous moduli of parts of the hPMN. Central regions stiffer than leading edge	Pa	AFM [91]	[57]
3	Elasticity and viscosity of the entire cell but with contact (E_inf) = 156 +/− 87	Pa	AFM	[87]
4	Elastic (storage) and viscous (loss) moduli of hPMNs from venous blood. Deformation can cause activation	dyn/cm^2	Particle tracking microrheology [92]	[53]
5	Deformability based on circularity: hPMNs from peripheral blood	Dimensionless	Hydrodynamic stretching	[47,81]
6	Deformability, chemotaxis correlation (1981) with fMLP concentrations. Increase in deformability with fMLP	Dimensionless	Elastometry (micropipette aspiration)	[80]
7	Transit time as read out of rigidity or stiffness of hPMN. Very good time resolution (1 s) for long time scale changes (minutes)	s	Single pore (5 μm) transit time analyzer	[38]
8	Transit time as a measure of deformability of hPMN	ms	Cell transit analyzer (8 μm diameter, 19 μm long)	[79]
9	Deformability as a function of apparent viscosity of hPMNs from ARDS, sepsis and septic shock patients	$dyn \cdot s/cm^2$	Micropipette aspiration (4–5 μm diameter)	[6]
10	Transit time through constrictions as a measure of deformability of resting and activated hPMN	s	MMM (5 μm, 7 μm), 189 constrictions	[14]

In addition to clarity in nomenclature, the very fact that resting or quiescent neutrophils can be activated by mechanical stimuli [53,59] calls into question those techniques that report passive mechanical properties of neutrophils in Table 4. Perhaps, different techniques perturb neutrophils differently, eliciting different neutrophil responses to such stimuli. Thus, the nature of neutrophil response to mechanical stimuli (neutrophil mechanotransduction) becomes a central issue in the interpretation of results obtained from the measurements of PMN mechanical properties, as well as any articulation of the physiological role of neutrophil mechanical properties. In Fig. 2, we give the timeline of neutrophil mechanotransduction, from the earliest report of PMN activation by mechanical stress [59], to the recent discovery of depriming of neutrophils by in-vivo-like stretching [14]. The physiological and pathophysiological implications of these findings will form the center of our discussion and their clinical relevance will be used to motivate further work in the concluding remarks. Before then, let us follow the humble neutrophil in its life journey from birth in the bone marrow to other compartments (Section 3), keeping track of cytoskeletal and mechanical alterations (in response to mechanical stimuli) that life in these compartments engender.

3. From bone marrow to blood and tissues

3.1. Mobilization and migration across BM endothelium

In post-natal humans, neutrophils are produced in the bone marrow (BM) as part of the blood-forming process termed hematopoiesis [2]. The antenatal umbilical cord serves a similar function as the bone marrow [93]. Every 24 h, about 10^{11} neutrophils are produced from hematopoietic stem cells (HSCs) under normal conditions [28,94]. They are then stored in the BM until homeostatic factors engender their release into the circulation (and if not released, BM macrophages phagocytose them [18]). To enter the vasculature, PMNs have to migrate across the BM endothelium, a process that is mediated by

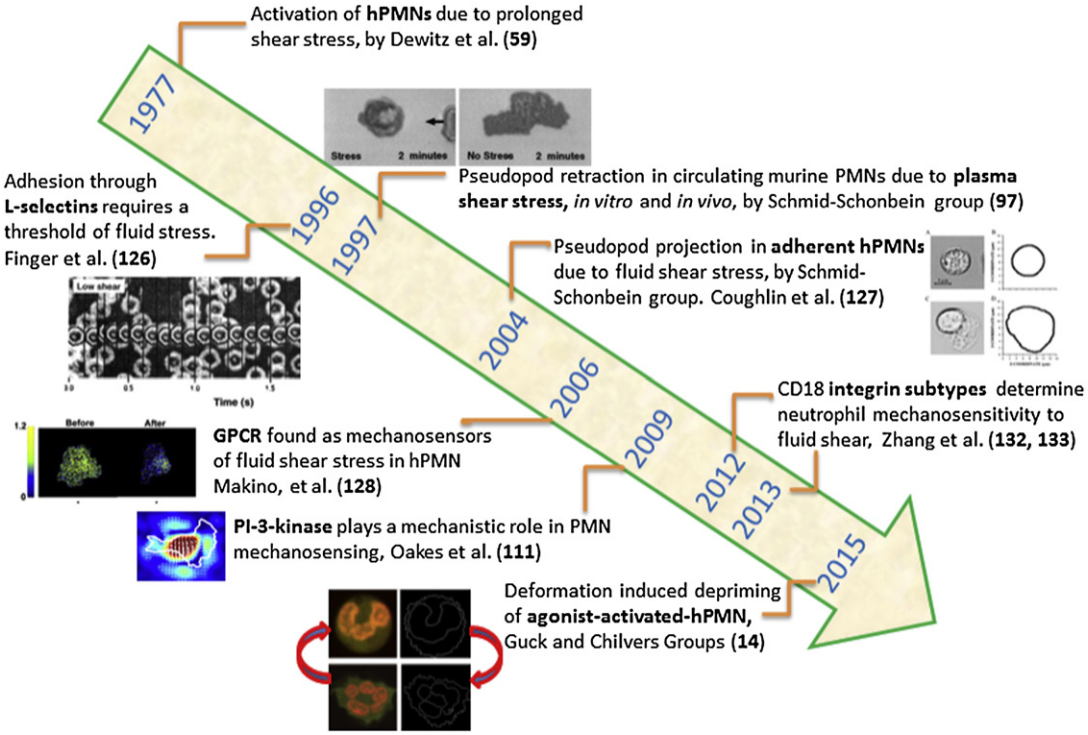

Fig. 2. Neutrophil mechanotransduction timeline from first report of mechanically induced activation to the discovery of deformation-induced depriming.

cytokines such as G-CSF which can activate neutrophils. But resting neutrophils do not migrate (see Table 1). From a mechanical perspective, a pertinent question here is whether mature BM neutrophils are always resting until primed or activated to migrate across the BM endothelium? There is a lack of data on hPMN activation before migration out of BM. If they get activated to enter circulation, when do they round up and become quiescent as we find them to be while in peripheral circulation? Yes, PMNs taken from peripheral blood are generally round and resting [16,56]. There should be in vivo priming/activation of PMNs from BM crossing the endothelium into circulation and *depriming* of those PMNs that have entered the circulation. Otherwise, how do we get the resting state in the blood? The 1996 discovery of in vitro depriming by a group led by Chilvers [56] opened up routes towards addressing this question. Moreover, there may be mechanical differences between BM PMNs and circulating PMNs even when both are in the resting state since they meet different environmental challenges. Such differences in mechanical properties have been found between murine BM derived PMNs and circulating murine PMNs [95]. The pathophysiological relevance of such differences is yet to be explored.

3.1.1. Mechanical differences between BM and circulating PMNs

An important challenge that would have to be overcome in exploring the biophysical differences between BM and circulating PMNs is the challenge of carrying out in vivo and in situ experiments on human subjects, for obvious reasons. Moreover, there are known differences between murine and human PMNs. For instance, murine PMNs do not produce defensins at all, while human PMNs do [26]. In mice, only 10–25% of circulating leukocytes are neutrophils while in humans 50–70% of circulating leukocytes are neutrophils [2]. The biophysical differences between BM and circulating PMNs will have to be explored in the larger context of possible neutrophil heterogeneity, which were mentioned in the introduction [26,28]. In Table 5, we show the various subsets of neutrophils, qualities that distinguish them, and the possible implications of such differences.

3.1.2. Depriming/deactivation vis-à-vis mechanics

Upon priming/activation (by soluble agonists), neutrophils exhibit a rapid and reversible fall in the high basal intracellular chloride (Cl-i) levels [58] that characterize resting neutrophils. Erythrocytes seemingly provide superoxide dismutase in vivo, to reduce neutrophil depriming by shear stress [96]. Neutrophils can undergo a cycle of complete priming, depriming, and repriming, as demonstrated using platelet activating factor (PAF) [56,16]. There are clear results showing that resting human neutrophils subjected to mechanical deformation can become activated, as evidenced by pseudopod formation within 1 or 2 min[53]. Furthermore, there are hints of depriming of neutrophils due to mechanical stress on the membrane [73]. Human neutrophils passed through 3 μm-wide and 9 μm-long polycarbonate pores under a constant pressure of 19.5 cm H_2O at 37 °C, and subsequently fixed at 15, 30, 60 and 120 s, showed an increasing recovery of F-actin content to within 95% of baseline resting values. Moreover, the distributions of F-actin at different time points post-filtration showed a corresponding movement of F-actin from a peripheral distribution characteristic of activated cells, to a more diffuse and interiorized distribution. The functional role of priming/depriming remains to be explored at least mechanistically, as highlighted previously [16].

3.2. Peripheral and pulmonary circulation

3.2.1. Priming and depriming under fluid shear stress

Instead of considering the resting state of neutrophils as a sort of steady-state condition, previous work reported that fluid shear stress offered by the plasma in circulation, helps to prevent circulating neutrophils (as well as monocytes and lymphocytes) from forming pseudopods [97]. The authors found that single human neutrophils and monocytes spreading on glass surfaces in vitro subjected to fluid shear stress of approximately 1 dyn/cm^2, showed an instantaneous retraction of pseudopods. This was reversible since removal of the fluid shear stress led to the return of pseudopod projection and cell spreading. Interestingly, retraction of pseudopods in response to shear stress could be suppressed by K^+ channel blockers and chelation of external Ca^{2+}[97]. In vitro experiments on human, rat, and mouse leukocytes and in vivo observations in the rat mesentery, soon provided evidence that inflammatory mediators such as platelet activating factor do suppress pseudopod retraction by fluid shear stress [98]. In a bid to unravel the molecular mechanisms for regulation of leukocyte response to fluid shear stress, the authors found that the shear stress response is enhanced by cGMP analogs, while the response is inhibited by blockers of soluble guanylate cyclase [98].

Schmid-Schönbein and co-workers have almost created a subdiscipline in neutrophil studies focused on unraveling the phenomenon of neutrophil response to fluid shear stress. They discovered that neutrophils adhering via β2-integrins exhibit a normal ability to project pseudopods, to migrate and to respond to fluid shear by rapid pseudopod retraction but neutrophils which adhere via β1-integrins become firmly adherent, show spreading, almost do not migrate and exhibit a significantly attenuated ability for pseudopod retraction under fluid shear [99]. Their results suggest that integrins play a regulatory role in the response of neutrophils to shear stress. They further investigated the kinetics of integrin adhesion molecules such as CD18 and found that fluid shear down-regulated CD18-associated immunofluorescence of extracellular epitopes, especially in areas of the membrane exposed to elevated levels of shear (1.5 dyn/cm^2 maximum shear stress; 1 dyn = 10 mN) [100]. At the same time, they showed that centrifugation (at 100, 600, or 900 g for 5, 10, 15, or 20 min) irreversibly attenuated neutrophils' ability to retract pseudopods in response to physiological shear stresses. Moreover, centrifuged neutrophils reintroduced into the rat circulation had an enhanced tendency to migrate into tissue [101]. Interestingly, glucocorticoids, which have anti-inflammatory activities on leukocytes, showed similar effect as high speed centrifugation, by reversing the normal fluid shear response (pseudopod retraction) both in vitro and in vivo [102]. That is, during shear exposure, neutrophils, monocytes and lymphocytes in whole blood (not separated in order to avoid additional centrifugation) exhibit pseudopod projection instead of retraction [102].

Table 5
Heterogeneity of neutrophils and possible implications.

Differences of neutrophils	Specification	Possible implications for
Marginated pool	In bone marrow, spleen, liver, lung	Control of tissue damage and microbial invasion [20,21,26]
Circulating pool	In bloodstream	Signaling between compartments, priming and depriming [22,23,26]
Age	Time in circulation	Maturation, differentiation [28,54]
Cell size	In diameter	Differentiation, activation stage [36,40,62]
Cell shape	Formation of pseudopods	Different capillary transit times, adhesion, (trans-) migration [4,35,69,99]
Nuclear size	In diameter	Different gene expressions, NET formation [25,55]
Nuclear shape	Banded/(multi-)lobulated	Maturation, differentiation [10,11]
Activation stage	In/decreased deformability	Different capillary transit times, (trans-)migration, phagocytosis [6,10,30,46,77]
Surface markers	e.g. CD18, CD66a, CD66b	Differentiation, signaling, adhesion, phagocytosis [15,21,23,26]

With this finding, the connection between pseudopod retraction (an aspect of depriming or deactivation) and resolution of inflammation (or at least anti-inflammatory effects) began to emerge.

Schmid-Schönbein's group, thus, worked on the hypothesis that deactivation in response to mechanical stimuli (shear stress) may be an important mechanism to decrease systemic inflammation and to reduce cardiovascular complications. So they kept fresh neutrophils in suspension, activated them with fMLP, maintained them in the suspended state without adhesion to endothelium, and sheared them in a cone-and-plate device. They found that a fraction of the activated neutrophils retracted their pseudopods under the influence of fluid shear and returned to round shape [96]. However, pseudopod retraction was observed only in the presence of erythrocytes (at shear stresses up to approximately 25 dyn/cm^2). To unravel the biochemical role of the erythrocytes, they observed that addition of superoxide dismutase (SOD) in phosphate buffer served to enhance neutrophil de-activation by fluid shear, leading to the suggestion that erythrocytes contribute to neutrophil de-activation by reducing the superoxide level in plasma [96].

3.2.2. Priming and depriming by other mechanical stimuli (constriction-induced stresses)

With billions of capillaries in the human lungs [65], millions of alveoli [65] and the fact that any blood cell, thus also a neutrophil, encounters about 40–100 capillary segments while being advected from an arteriole to a venule via pulmonary capillaries, it is clear that the pulmonary microcirculation provides another mechanical stimulus to circulating cells- via capillary constrictions smaller than the average diameter of blood cells (see Table 2). The physiological benefits of having the lungs as a site of depriming have already been postulated [56,77] and the consequences for pathology hypothesized [16,103]. In fact, contemporaneous with our unpublished data showing that mechanical deformation can deprime soluble agonist-activated neutrophils [14] there is emerging clinical evidence that the healthy human lungs selectively retains primed neutrophils in order to deprime them and re-release them into the systemic circulation as resting cells [104]. This recent work suggested that the yet-to-be-identified physiological depriming mechanism(s) may fail in patients with ARDS, resulting in increased numbers of primed neutrophils within the systemic circulation [104]. It is important to note that even in the circulatory phase of neutrophils, there could be differences in mechanosensitivity between advecting PMNs and the so-called marginated pool [69] implicated in several lung diseases. Obviously, finding the physiological depriming mechanism(s) is a biophysical grand challenge with potential clinical impact in pulmonary, inflammatory as well as circulatory disorders and beyond. In addition to mechanical stimulation by fluid shear stresses and capillary constriction imposed strains on PMNs in the circulatory phase of their lifespan, the ECM and other cells impose strains on PMNs following their extravasation from the vasculature to tissues.

3.3. From circulation to tissues

3.3.1. Deactivation and reactivation by cell-substrate (matrix) stresses

Neutrophils in the circulation receive priming or activating signals [17], roll [35], tether [17,35,105], and cross the endothelium in a set of complex steps [17,26,106] beyond the scope of this review. Blood vessels have been modeled in vitro using fibronectin-coated polyacrylamide gels of varying stiffness to investigate the effect of substrate-stiffness on neutrophil extravasation [107] but it is not clear whether the observed increase in transmigration though the endothelial cells on stiff substrates is due directly to mechanosensitivity of the neutrophils. Since the recent work led by Aranda-Espinoza is perhaps the first exploration of the effects of substrate stiffness on neutrophil transmigration [107], it is reasonable to expect that studies on how PMNs sense the forces and constraints imposed on them by the endothelial wall will follow. Already, the Aranda-Espinoza group has reported that over 93% of human PMNs preferentially exploit the paracellular mode of transmigration in their in vitro model [39]. Considering the report that an actively transmigrating PMN exerts forces three times higher than those of activated and adherent PMN that are not transmigrating [108], we reason that transmigrating PMNs would rather not be 'distracted' by mechanical constraints imposed by the endothelium, but would forge ahead into tissues towards the sites where immune duties call.

Neutrophils cross the vascular endothelium to extravascular tissues, following chemotactic gradients [36] to effect repair of injured but sterile tissue [24], fight bacterial infections [33] or engage in pro-inflammatory activities [109]. They get exposed to regions with differing mechanical properties, which cells in general can also actively sense [49] and respond to [51,110]. This is a different form of mechanosensitivity, and contrasts to the situation where cells are being deformed by external forces. It has been shown that all cells do not respond in the same way to matrix stiffness [111]. In fact, neutrophils seem not to respond to stiffness differences that affect other cell types [112]. Of course, this does not mean that PMNs migrating in tissues are not affected in any way by the differences in the mechanical properties of the ECM. In fact, they are [111]. Rather, activated PMNs need to behave reliably in many different tissues during directed migration towards sites of injury or infection. No wonder they display a diverse repertoire of methods for generating directional motion [113]. A pertinent question here is whether there are in tissues mechanical stimuli analogous to the fluid shear stresses and the capillary constriction-induced cytoskeletal deformations that are now known to lead to PMN priming and depriming? After all, mechanotransduction of interstitial fluid stresses have been reported to govern 3D migration of mammary adenocarcinoma cells [114]. We speculate that PMNs heading to foci of infection or injury may not be deprimed by interstitial fluid pressure but PMNs in pleural effusions or in the cerebrospinal fluid may be influenced by such fluid stresses. Lastly, what about reverse migration of some PMNs back into the circulation?

The reverse migration of tissue neutrophils back into the circulation [115] is a new finding that may lead to paradigm shifts in neutrophil biology. It was first reported in in vitro assays. Advances in intravital imaging enabled the confirmation of reverse neutrophil migration in zebra fish and mice. Blood goes round the human body in a matter of minutes. If neutrophils at sites of tissue infection reverse-migrate into the circulation, then several bacterial and viral pathogens that invade neutrophils, highjack or compromise their bactericidal abilities, can understandably spread around the body and into distal organs in a matter of minutes. It remains to be known whether the cytoskeletal changes induced in leukocytes during infection [116] can promote or reduce neutrophil migration in tissue or advective ability in blood.

4. Discussion

4.1. Morphometric and rheological controversies

Measurements of resting and activated PMN size done on fixed cells and on cells in suspension yielded results significantly less than those performed on neutrophils in blood smears [42,67,69]. These earliest measurements reported PMN diameter as less than 10 μm. The often-cited work led by Downey in 1989 [42] gave 8 μm as the diameter of activated cells. However, more recent measurements have yield sizes between 7 and 11 μm, with activated cells slightly larger than resting cells [14,37,40,62] (see Table 2). An important source of differences seems to be the fact that the PMN has numerous fine membrane foldings that leave the cell with a membrane area much larger than needed to contain the cell volume [29,67,117]. While the shrinkage of this excess membrane in hypertonic environment, for instance, may account for some of the variations in morphometry results, an important mechanotransduction reason for future work to delve into more accurate assessment of PMN excess membrane includes the implications of

the latter in sensing mechanical stimuli, especially fluid shear stress. Moreover, an increase in cell volume between resting and activated PMNs presents cell size as a plausible reason for PMN sequestration in the lungs of patients of pulmonary diseases [42,118] and other conditions. Of course, the rheological difference between resting and activated PMNs remains another major reason for such sequestration. Yet, rheological results are even more controversial than the morphometric ones.

A major finding, that soluble agonist-activated or primed neutrophils are stiffer than resting neutrophils [14,42,73,118,119] and that such stiffening is a necessary and sufficient condition for sequestration in capillaries [42] has been challenged recently [47,81]. Gossett et al. imposed very high strains on the cells in their version of the deformability cytometer [81] (DC) and found agonist-activated PMNs to be more deformable than resting PMNs. It is known that such high strains can fluidize F-actin [120,121]. Otto et al. [47], in introducing RT-DC, also found agonist-activated PMNs to be more deformable than resting PMNs. Interestingly, both techniques measure cell mechanics at very short time scales: milliseconds. Can it be that activated PMNs are stiffer at longer timescales of seconds and minutes, timescales which are physiologically more relevant to PMNs in vivo (see Table 3)? The jury is still out on this controversy and clinicians need a resolution. Thus, the rheological controversy itself constitutes a biomedical challenge discussed below. Before then, we give a broader perspective to the issue, arising from the danger of extrapolating possibly convoluted in vitro results to in vivo situations especially in the case of PMNs.

4.2. Impact of methods on results

It is already known that certain methods of isolating neutrophils from the blood inadvertently leave them activated [122]. Measurement techniques may actually alter neutrophil states. Of course, one can intentionally use mechanical stimulus or other stimuli imparted by measurement probes, to investigate neutrophils. However, when inadvertent, the interpretation of results might be misleading. The use of micropipette aspiration in particular, in the era when reversible priming [56] was often not accounted for, calls for caution when looking back at such results, for instance, the report that neutrophils in patients with sepsis, septic shock and ARDS are less deformable [6]. There is an important interplay between cell mechanics and mechanosensing in the case of neutrophils.

Generally, if one is measuring passive cell mechanics it is important that the cell actually stays passive. For many cell types this is a good assumption. However, the situation changes drastically with neutrophils. These cells are professional surveillance cells. They are, as we have summarized in this review, exquisitely sensitive to many stimuli, including cell-substrate contact or adhesion and even to rather gentle mechanical perturbation. How, then, can one measure neutrophil mechanics without also inducing priming/activation of the cell, which in turn alters its mechanical properties? In this regard, neutrophils, due to their mechanosensitivity, are probably the most difficult cell type to characterize mechanically. In no other cell type is cell mechanics and mechanosensitivity so closely intertwined as in neutrophils. No wonder they have been described as experimentally intractable [21]. It is then not surprising that there are rather conflicting reports in the literature where the mechanosensitive response of neutrophils is not taken into account. Magnitude, spatial extent and duration of mechanical stress can all elicit different measurement outcomes. For example, if the mechanical characterization is over before the cell has time to alter its mechanical properties (for instance, milliseconds in deformability cytometers) then the result will likely differ from measurement techniques where cells are probed over seconds or minutes (AFM, optical stretcher, micropipette aspiration) and the cell has time to alter its mechanical properties. Another distinction can come from the presence of mechanical contact with surfaces (AFM, micropipette aspiration) where adhesion molecules might be involved in a mechanosensitive response, in contrast to techniques without such contact (OS, DC, RT-DC) where instead stress-activated ion channels play a more dominant role.

Perhaps passive neutrophils (in the classical sense of passive materials) do not exist. Of course, we do not mean dead neutrophils. Neutrophils deformed into a sausage shape in a glass micropipette recovered their normal spherical shape in 1 min after expulsion from the micropipette [76]. Those neutrophils held only for 5 s or less in the micropipette underwent initial, rapid elastic rebounds [76]. There is convincing evidence that resting human neutrophils subjected to large mechanical deformation exhibit a rapid decrease and then recovery in F-actin content and distribution. But the pattern of changes is quite complex: no change under small-scale deformation (5 μm pores), decrease with large-scale deformation (3 μm pores) under high driving pressure (19.5 cmH_2O) but increase in F-actin for low driving pressure (5 cmH_2O) [73]. The initial rapid reduction in both storage and loss moduli following passage of human resting neutrophils through narrow capillary-sized pores reported in [53] seems to be due to (at least partially) the aforementioned initial rapid depolymerisation of F-actin [73]. It is noteworthy that the rapid reduction in moduli was followed by recovery to near initial values within 1 min after stimulation, thereby correlating with a recentralization of actin distribution [53,73]. Recent advances in intravital imaging may allow the investigation of the in vivo relevance of these dynamic rheological properties of neutrophils, at least in mice. Along this line, mobile, extravascular neutrophils with rounded morphologies have, surprisingly, been identified [75]. To further complicate matters, biphasic responses are possible. For instance, pentoxifylline alters PMN deformability in a biphasic manner: enhancement at lower concentration and suppression at higher concentration [123].

Mechanical properties and mechanotransduction are not the only aspects of neutrophil studies that can be altered and convoluted by methods of probing. Even the very lifespan of neutrophils is a subject of controversies largely due to differences in method of determination. Mature PMNs were classically regarded to be short-lived (within a day) in the circulation [22]. But a group claimed that neutrophils can last up to 5.4 days [124] in the circulation, though the same group has recently given a lower figure. The classic position was based on ex vivo labeling, reintroduction and monitoring of neutrophils. The longer lifespan was obtained by in vivo labeling! Convoluted results notwithstanding, there is clear evidence that certain mechanical stimuli can prime resting PMN and deprime agonist-activated PMNs in vitro [14,52,97]. The molecular mechanisms behind such peculiar mechanotransduction in PMNs are next discussed, with in vivo implications in mind.

4.3. Molecular mechanism(s) of mechanotransduction in PMNs

It is reasonable to expect a mechanism by which mechanical stimuli such as fluid shear stress (Fig. 3) and repeated mechanical stretching do not only lead to priming/activation, which has been shown in several experiments, but also to de-priming/de-activation. Otherwise all neutrophils in circulation, or at least for a considerable time after they first get into circulation, would be primed/activated and overly sensitive to further inflammatory signals. Using clinical data in the context of inflammatory bowel disease (IBD) and mathematical modeling, Summers et al. argued that since only 4% of circulating PMNs migrate into an inflamed bowel segment, it means that if the 96% of non-migrating cells exit in a primed state, then at steady state >90% of circulating neutrophils would be primed if no depriming took place [125]. They concluded that because the highest level of priming seen in IBD is ~40%, this indicates that depriming within the circulation must take place. But how? Fluid shear stress [70,96] and repeated stretching by capillary constrictions [14] (stenotic stress) would appear to be strong candidates for the role (Fig. 3). We now review the molecular mechanisms postulated to account for this mechanotransduction.

The mechanical stimuli or specifically stress components may be acting on the cell cytoplasm or on the plasma membrane. On the surface

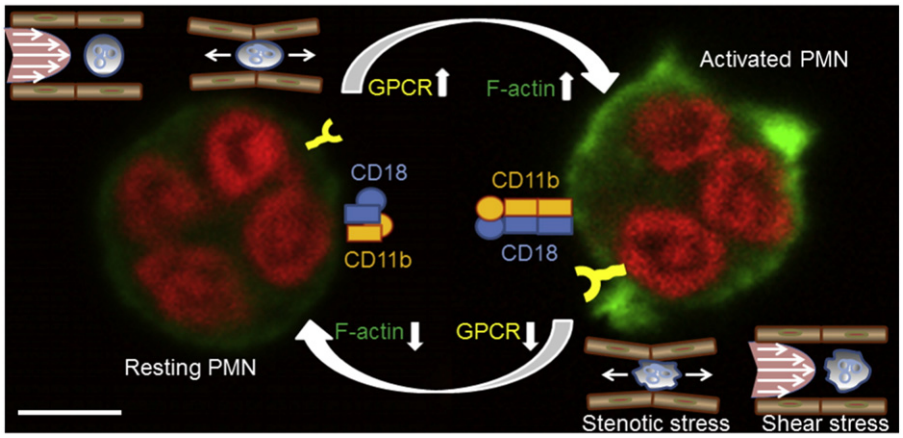

Fig. 3. Putative molecular mechanism(s) of mechanotransduction in PMN activation and deactivation. Fluid shear stress (1–10 dyn/cm^2) and capillary-constriction imposed stress (stenotic stress) can activate resting PMN with GPCRs serving as mechanosensors and leading to enhanced expression of integrins (CD18) on the surface of activated PMNs. Already activated PMNs can also be deactivated by fluid shear and stenotic stresses, with the former acting on the cell membrane via GPCRs without any deformation of the cytoskeleton. The latter involves cytoskeletal deformation. The confocal images, which are illustrative of activation/deactivation, show Syto 61 (red nuclear dye) and Alexa Fluor-Phalloidin stained PMNs indicating F-actin distribution (green) in resting and activated neutrophils. The scale bar is 5 μm.

of a plasma membrane, the stress may be acting on molecular attachments via membrane adhesion molecules such as integrins and cadherins, or the stress may be generated by fluid flow over the membrane. The fluid shear stress mechanotransduction reported extensively by the Schmid-Schönbein group has been presented as a control mechanism that neutrophils use in reducing the formation of pseudopods and the surface expression of CD18 integrins, thereby rendering the cells rounded, deformable, and non-adhesive. Specifically, using antibodies against human neutrophil β1 and β2 integrins, it was found that neutrophils adhering via β2 integrins retracted pseudopods in response to fluid shear and migrated normally, but those adhering via β1 integrins showed no migration, attached firmly and had attenuated response to fluid shear [99]. The authors suggested that their finding could be a mechanism to prevent PMN spreading on the endothelium, thereby facilitating their passage through the circulation. However, this does not explain how activated PMNs in suspension in the peripheral blood might get deprimed. Moreover, L-selectins and certain shear thresholds were hitherto found to promote and maintain rolling interactions of neutrophils with the endothelium [126]. Closer to explaining pseudopod retraction in suspended and activated PMNs was the discovery of the downregulation of CD18 molecules on activated and suspended PMNs due to physiological levels of fluid shear [100]. The authors deduced that fluid shear stresses caused release of cysteine protease(s) including cathepsin B, followed by the cleavage of extracellular domains of CD18 molecules and possible membrane detachment. Interestingly, they suggested that fluid shear in the circulation might cause cleavage of CD18 molecules leading to anti-inflammatory effects (depriming) as well as causing redistribution of CD18 molecules in ways that actually enhance inflammatory reaction [100]. Not surprising was the follow-up finding that resting adherent cells in vitro showed pseudopods in response to fluid shear [127]. This ability of fluid shear stress to cause activation of resting PMNs and deactivation of already activated PMNs via transmembrane receptors are illustrated in Fig. 3.

G-protein coupled receptors (GPCR) have also been described as mechanosensors of fluid shear stress in HL60-derived neutrophils [128]. The authors specifically targeted one GPCR, the formyl peptide receptor (FPR). They found that undifferentiated HL60 cells which lack FPR formed few pseudopods and showed no detectable response to fluid shear stress. In contrast, both in DMSO-differentiated HL60 cells and in undifferentiated cells made to express FPR by transfection, there was pseudopod projection with fMLP treatment and robust pseudopod retraction following fluid shear stress [128]. In summary, both demonstrated and suggested molecular mechanisms behind PMN activation and deactivation in response to fluid shear stress were reviewed in 2007 [52]. Prominent highlights of this review include the assertion that physiological levels of shear stresses involved range between 1 and 10 dyn/cm^2, which is insufficient to cause deformation of the cytoskeleton itself. Their effect is only on the cell membrane. Furthermore, it was suggested that GPCR down-regulation by fluid shear and F-actin depolymerization, *inter alia*, lead to pseudopod retraction while proteolytic cleavage of β2 integrins cause membrane detachment [52] (Fig. 3). Incidentally, the aforementioned molecules are among the key components of the emerging concept of mechanical homeostasis, a framework for giving mechanistic insights to mechanotransduction from molecules to cells and tissues [129]. Work done since then (2007) on PMN mechanotransduction [130,131] has not provided conclusive evidence for these suggested mechanisms for fluid shear-induced mechanotransduction in PMNs, but has ramified the implications (for instance, evidence that the CD18-cleavage responses of neutrophils to shear interfere with fibrinogen binding and platelet adhesion [132] and is dependent on CD18 integrin sub-types [132,133]). Moreover, experimental systems have been devised for nanoscale study of the interactions between cell membrane receptors and biofunctionalized surfaces in the context of cell mechanics [134]. Such biomimetic systems can be extended to mechanotransduction studies in neutrophil priming and depriming. Furthermore, depriming by stresses which deform the cytoskeleton (such as stenotic stress depicted in Fig. 3) has been discovered [14].

The capillary constriction-like stretches imposed by the optical stretcher and microfluidic microcirculation mimetic clearly do act on the cytoplasm or specifically, the cytoskeleton, in addition to the cell membrane. The molecular mechanism is not yet clear. But it supports clinical data adducing to the physiological role of depriming, with pathophysiological consequences [104].

4.4. Biomedical and clinical challenges

Over two decades ago, Worthen et al. showed that the retention of neutrophils in pulmonary capillaries is caused by the stiffening of stimulated or activated neutrophils [42] as noted above. It was argued that compared to systemic vascular beds, the initial sequestration of neutrophils in the pulmonary microcirculation is more dependent on mechanical forces than adhesion-mediated rolling along the endothelial surfaces [75], based on results in [17]. The sequestration of activated murine PMNs in pulmonary capillaries has been shown to occur independent of selectin-mediated rolling and cytoskeletal rearrangements,

evaluated by sub-membrane F-actin rims, interpreted as inductive of stiffening [118]. The claim was that such neutrophils may not pass through capillaries. However, these results and claims have yet to be replicated in vivo in humans.

In clinical settings, the neutrophil's antibacterial and antifungal abilities seemingly turn into liabilities by leading to or aggravating COPD, bronchiectasis, cystic fibrosis, and certain forms of asthma [15]. Single intra-venal infusion of 0.25 mg TPA led to an increase in the neutrophil count in patients suffering neutropenia following tumor-related chemotherapy [135]. In human and murine atherosclerotic lesions, neutrophils have been detected and been linked to pathophysiology [136]. The neutrophils of multiple sclerosis patients tend to stay in a primed state based on indicators of priming including reduced apoptosis, higher expression of TLR-2, fMLP receptor, IL-8 receptor and oxidative burst as well as higher levels of NETs in serum [137]. As earlier observed [15], a key challenge in conditions such as COPD, bronchiectasis, cystic fibrosis, and certain forms of asthma is to find a way of manipulating neutrophil function without compromising their antibacterial and antifungal capacity. Thus, we now discuss some pharmacological interventions that have bearing on PMN mechanotransduction and rheology.

Human PMNs from patients of diabetes have been found to be more rigid than those from healthy patients [30]. A recent (2013) prospective study of the anti-inflammatory effects of certain drugs went as far as using loss of deformability, morphological changes, and increase in neutrophil elastase as measures of neutrophil activation due to incubation with the pro-inflammatory cytokine interleukin-8 [79]. Change in deformability, investigated with a cell transit analyzer, was one of the three markers of activation used in the study. It is very instructive to note that the drugs milrinone, piclamilast, urinastatin, ketamine, protein C concentrate and FK 409 show deactivating effects on activated neutrophils. The deactivating effects consisted in (1) improved deformability, (2) reduced pseudopod formation and the release of (3) neutrophil elastase [79]. Perhaps, these drugs may deprime PMNs and thereby solve the problem of stiffening and retention in capillaries of patients of circulatory and inflammatory disorders. Perhaps, these drugs may deprime PMNs and hence solve the problem of impaired shear stress induced deactivation [52] or impaired constriction-induced depriming [14,104]. Instructively, established anti-inflammatory drugs such as lipocortin have been found to alter PMN deformability [138]. It is not clear what effects these drugs have on PMN mechanotransduction. Now that transcriptional profile changes during the transition of PMNs from the bone marrow, to the circulation and to sites of infection have been reported [139], it should be interesting to investigate the effects of these anti-inflammatory drugs on the transcriptional profile, the cytoskeletal makeup and the mechanosensitivity of PMNs. It is already fascinating that among the 360 genes upregulated in BM PMN, downregulated in circulating PMN and upregulated in PMNs in infected tissue, are those genes responsible for a similar up-down-up-regulation of the actin cytoskeleton [139].

It is of interest that PMNs of patients with multiple sclerosis, who suffer from inflammation, stayed primed [137], whereas patients with diabetes, who were long-term immunocompromised, have more rigid PMNs [30]. These observations regarding different mechanical phenotypes of PMNs might help to explain yet unknown links between immunodysfunction and certain disease characteristics in future.

Exploring the lung-transit – as an inherent depriming mechanism of activated PMNs to balance the immune surveillance of circulating neutrophils – opens a new understanding of inflammation and infection control. As the lung serves as a natural regulation system of neutrophil activation, one could envision therapeutic options to assist this mechanism when it fails. Drugs with depriming effects on neutrophils could interfere with overwhelming inflammation in patients with ARDS as well as systemic inflammatory response syndrome (SIRS) or septic shock. Notably, depriming effects on neutrophils could be shown for established anti-inflammatory agents, such as lipocortin/annexin. Depriming of neutrophils might therefore be one of the beneficial aspects of glucocorticoids, which are already applied to patients with severe inflammation and circulatory problems as well as to patients with autoimmune diseases. As discussed in this review, the techniques to measure PMN activation/deactivation have remarkably improved. By mechanical phenotyping of PMNs, e.g. using a high-throughput analysis with RT-DC, one might screen the priming/depriming effects of established as well as promising new drugs on PMNs. One could speculate that monitoring PMN activation/deactivation in patients with autoimmune inflammation or severe sepsis could assist in anti-inflammatory treatment strategies.

5. Concluding remarks

Neutrophils produced in the BM presumably become primed or activated as they migrate across the BM endothelium into the circulation. Priming and activation involve cytoskeletal reorganization which leads to changes in the mechanical properties of neutrophils. Studies on mechanical properties of neutrophils in the context of priming are usually undertaken using blood neutrophils from the circulating pool. Further work is required to characterize the mechanical properties of BM-derived neutrophils, and their response to mechanical stimuli. Likewise, there are known biochemical differences between resting circulating PMNs and resting BM neutrophils. There is need for further rheological characterization of both resting and activated BM neutrophils as well as resting and activated circulating neutrophils. Impaired recruitment, mobilization, homing and clearance of neutrophils in the BM are among the health and disease related issues that may be better understood through further rheological characterization and assessment of mechanosensitivity.

In the blood, circulating neutrophils differ from the marginated pool at the level of gene expression, protein synthesis, surface markers, adhesion molecules and signaling. While much rheological work has been done on circulating neutrophils with a view to resolving several questions about neutrophil sequestration in the pulmonary microcirculation, quite little is known about the mechanical properties of the marginated pool. A very interesting implication of current neutrophil mechanotransduction results (mechanically induced depriming via fluid shear stresses and via deformation caused by capillary-like constrictions) is that the lungs, which have the largest reservoir of marginated neutrophils, may actually serve the purpose of collecting activated circulating neutrophils, depriming them though mechanical stimuli and releasing them back into circulation. Understanding the molecular mechanism(s) behind deformation or constriction-induced depriming may lead to new diagnostic insights and therapeutic options for inflammatory, cardiocirculatory and auto-immune disorders such as ARDS, COPD, artherosclerosis and diabetes.

Succinctly, the connection between cell mechanics, controlling the passage of cells through the microcapillary bed of the lungs for example or the diapedesis and migration through tissues and the mechanosensitivity of neutrophils upon deformation, which in turn can lead to alterations in exactly these cell mechanical properties provides a fascinating and rich feedback-system that has only just begun to be explored. With this we serve a “call to arms” for both fields – cell mechanics and mechanotransduction – to lock arms and explore. The outcome could be very important new insights that can expand our knowledge about neutrophil function and malfunction, broaden our understanding of the human immune system in health and disease with implications for etiologic, diagnostic and therapeutic considerations in multiple human diseases.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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References

[1] M.S. Campbell, M.A. Lovell, G.J. Gorbsky, Stability of nuclear segments in human neutrophils and evidence against a role for microfilaments or microtubules in their genesis during differentiation of HL60 myelocytes, J. Leukoc. Biol. 58 (1995) 659–666.
[2] N. Borregaard, Neutrophils, from marrow to microbes, Immunity 33 (2010) 657–670.
[3] J. Ziaber, et al., The immunoregulatory abilities of polymorphonuclear neutrophils in the course of multiple sclerosis, Mediat. Inflamm. 7 (1998) 335–338.
[4] C.M. Doerschuk, N. Beyers, H.O. Coxson, B. Wiggs, J.C. Hogg, Comparison of neutrophil and capillary diameters and their relation to neutrophil sequestration in the lung, J. Appl. Physiol. 74 (1993) 3040–3045.
[5] B. Alberts, et al., Molecular Biology of the Cell, 4th ed. Garland Science, 2002.
[6] G.M. Athanassiou, et al., Neutrophil deformability in patients with sepsis, septic shock, and adult respiratory distress syndrome, Crit. Care Med. 28 (2000) 3–7.
[7] G.B. Nash, H. Meiselman, Rheological properties of individual polymorphonuclear granulocytes and lymphocytes, Clin. Hemorheol. 6 (1986) 87–97.
[8] G.W. Schmid-Schönbein, K. Sung, H. Tözeren, Passive mechanical properties of human leukocytes, Biophys. J. 36 (1981) 243–256.
[9] E. Evans, B. Kukan, Passive material behavior of granulocytes based on large deformation and recovery after deformation tests, Blood 64 (1984) 1028–1036.
[10] P. Ruef, E. Zilow, O. Linderkamp, Deformability of peripheral immature and mature neutrophils, Clin. Hemorheol. 14 (1994) 233–236.
[11] O. Linderkamp, P. Ruef, B. Brenner, Passive deformability of mature, immature and active neutrophils in healthy and septicemic neonates, Pediatr. Res. 44 (1998) 946–950.
[12] E. Feuk-Lagerstedt, E.T. Jordan, H. Leffler, C. Dahlgren, A. Karlsson, Identification of CD66a and CD66b as the major galectin-3 receptor candidates in human neutrophils, J. Immunol. 163 (1999) 5592–5598.
[13] T. Schmidt, et al., CD66b overexpression and homotypic aggregation of human peripheral blood neutrophils after activation by a gram-positive stimulus, J. Leukoc. Biol. 91 (2012) 791–802.
[14] A. Ekpenyong et al., Mechanical deformation induces depriming of neutrophils, Unpubl data, 2015.
[15] A.S. Cowburn, A.M. Condliffe, N. Farahi, C. Summers, E.R. Chilvers, Advances in neutrophil biology: clinical implications, Chest 134 (2008) 606–612.
[16] A.M. Condliffe, E. Kitchen, E.R. Chilvers, Neutrophil priming: pathophysiological consequences and underlying mechanisms, Clin. Sci. (Lond.) 94 (1998) 461–471.
[17] A.R. Burns, C.W. Smith, D.C. Walker, Unique structural features that influence neutrophil emigration into the lung, Physiol. Rev. 83 (2003) 309–336.
[18] O. Leavy, Haematopoiesis: neutrophil clearance keeps the beat, Nat. Rev. Immunol. 13 (2013) 470.
[19] S.F. Van Eeden, M.E. Klut, B.A. Walker, J.C. Hogg, The use of flow cytometry to measure neutrophil function, J. Immunol. Methods 232 (1999) 23–43.
[20] B. McDonald, P. Kubes, Neutrophils and intravascular immunity in the liver during infection and sterile inflammation, Toxicol. Pathol. 40 (2012) 157–165.
[21] B. Amulic, C. Cazalet, G.L. Hayes, K.D. Metzler, A. Zychlinsky, Neutrophil function: from mechanisms to disease, Annu. Rev. Immunol. 30 (2012) 459–489.
[22] H.L. Wright, R.J. Moots, R.C. Bucknall, S.W. Edwards, Neutrophil function in inflammation and inflammatory diseases, Rheumatology (Oxford) 49 (2010) 1618–1631.
[23] A. Mantovani, M.A. Cassatella, C. Costantini, S. Jaillon, Neutrophils in the activation and regulation of innate and adaptive immunity, Nat. Rev. Immunol. 11 (2011) 519–531.
[24] C. Nathan, Neutrophils and immunity: challenges and opportunities, Nat. Rev. Immunol. 6 (2006) 173–182.
[25] V. Brinkmann, A. Zychlinsky, Beneficial suicide: why neutrophils die to make NETs, Nat. Rev. Microbiol. 5 (2007) 577–582.
[26] E. Kolaczkowska, P. Kubes, Neutrophil recruitment and function in health and inflammation, Nat. Rev. Immunol. 13 (2013) 159–175.
[27] M. Phillipson, P. Kubes, The neutrophil in vascular inflammation, Nat. Med. 17 (2011) 1381–1390.
[28] S. Bekkering, Another look at the life of a neutrophil, World J. Hematol. 2 (2013) 44.
[29] L.M. Pierini, et al., Membrane lipid organization is critical for human neutrophil polarization, J. Biol. Chem. 278 (2003) 10831–10841.
[30] Z. Pécsvarády, et al., Decreased polymorphonuclear leukocyte deformability in NIDDM, Diabetes Care 17 (1994) 57–63.
[31] R.A. Jannat, G.P. Robbins, B.G. Ricart, M. Dembo, D.A. Hammer, Neutrophil adhesion and chemotaxis depend on substrate mechanics, J. Phys. Condens. Matter 22 (2010) 194117.
[32] T.C. Barnes, M.E. Anderson, S.W. Edwards, R.J. Moots, Neutrophil-derived reactive oxygen species in SSc, Rheumatology (Oxford) 51 (2012) 1166–1169.
[33] V. Brinkmann, et al., Neutrophil extracellular traps kill bacteria, Science 303 (2004) 1532–1535 (80-).
[34] T. Lämmermann, et al., Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo, Nature 498 (2013) 371–375.
[35] E. Finger, R.E. Bruehl, D.F. Bainton, T.A. Springer, Differential Role for Cell Shape in Neutrophil Tethering and Rolling, J. Immunol. 157 (1996) 5085–5096.
[36] E. Fernández-Segura, J.M. García, J.L. Santos, A. Campos, Shape, F-actin, and surface morphology changes during chemotactic peptide-induced polarity in human neutrophils, Anat. Rec. 241 (1995) 519–528.
[37] G.I. Ruban, S.M. Kosmacheva, N.V. Goncharova, D. Van Bockstaele, V.A. Loiko, Investigation of morphometric parameters for granulocytes and lymphocytes as applied to a solution of direct and inverse light-scattering problems, J. Biomed. Opt. 12 (2013) 044017.
[38] R.S. Frank, Time-dependent alterations in the deformability of human neutrophils in response to chemotactic activation, Blood 76 (1990) 2606–2612.
[39] K.M. Stroka, H.N. Hayenga, H. Aranda-Espinoza, Human neutrophil cytoskeletal dynamics and contractility actively contribute to trans-endothelial migration, PLoS One 8 (2013) e61377.
[40] E.B. Lomakina, C.M. Spillmann, M.R. King, R.E. Waugh, Rheological analysis and measurement of neutrophil indentation, Biophys. J. 87 (2004) 4246–4258.
[41] S. Paschke, et al., Technical advance: inhibition of neutrophil chemotaxis by colchicine is modulated through viscoelastic properties of subcellular compartments, J. Leukoc. Biol. 94 (2013) 1091–1096.
[42] G.S. Worthen, B. Schwab, E.L. Elson, G.P. Downey, Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries, Science 245 (1989) 183–186.
[43] M. Zhang, et al., Quantitative characterization of viscoelastic properties of human prostate correlated with histology, Ultrasound Med. Biol. 34 (2008) 1033–1042.
[44] J. Guck, et al., Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence, Biophys. J. 88 (2005) 3689–3698.
[45] T.W. Remmerbach, et al., Oral cancer diagnosis by mechanical phenotyping, Cancer Res. 69 (2009) 1728–1732.
[46] J. Guck, E.R. Chilvers, Mechanics meets medicine, Sci. Transl. Med. 5 (2013) 212fs41.
[47] O. Otto, et al., Real-time deformability cytometry: on-the-fly cell mechanical phenotyping, Nat. Methods 12 (2015) 199–202.
[48] D. Di Carlo, A mechanical biomarker of cell state in medicine, J. Lab. Autom. 17 (2012) 32–42.
[49] T. Iskratsch, H. Wolfenson, M.P. Sheetz, Appreciating force and shape — the rise of mechanotransduction in cell biology, Nat. Rev. Mol. Cell. Biol. 15 (2014) 825–833.
[50] D. Ingber, Mechanobiology and diseases of mechanotransduction, Ann. Med. 35 (2003) 564–577.
[51] S. Kumar, Cellular mechanotransduction: stiffness does matter, Nat. Mater. 13 (2014) 918–920.
[52] A. Makino, et al., Mechanotransduction in leukocyte activation: a review, Biorheology 44 (2007) 221–249.
[53] B. Yap, R.D. Kamm, Mechanical deformation of neutrophils into narrow channels induces pseudopod projection and changes in biomechanical properties, J. Appl. Physiol. 98 (2005) 1930–1939.
[54] D. El Kebir, J. Filep, Targeting neutrophil apoptosis for enhancing the resolution of inflammation, Cells 2 (2013) 330–348.
[55] X. Zhang, et al., Gene Expression in Mature Neutrophils: Early Responses To Inflammatory Stimuli, J. Leukoc. Biol. 75 (2004) 358–372.
[56] E. Kitchen, A.G. Rossi, A.M. Condliffe, C. Haslett, E.R. Chilvers, Demonstration of reversible priming of human neutrophils using platelet-activating factor, Blood 88 (1996) 4330–4337.
[57] Y.J. Lee, D. Patel, S. Park, Local rheology of human neutrophils investigated using atomic force microscopy, Int. J. Biol. Sci. 7 (2011) 102–111.
[58] S. Busetto, E. Trevisan, E. Decleva, P. Dri, R. Menegazzi, Chloride movements in human neutrophils during phagocytosis: characterization and relationship to granule release, J. Immunol. 179 (2007) 4110–4124.
[59] T. Dewitz, R. Martin, T. Hung, L. McIntire, Mechanical trauma in leukocytes, J. Lab. Clin. Med. 90 (1977).
[60] D.A. Fletcher, R.D. Mullins, Cell mechanics and the cytoskeleton, Nature 463 (2010) 485–492.
[61] L. Deng, et al., Fast and slow dynamics of the cytoskeleton, Nat. Mater. 5 (2006) 636–640.
[62] D.Y. Orlova, M.A. Yurkin, A.G. Hoekstra, V.P. Maltsev, Light scattering by neutrophils: model, simulation, and experiment, J. Biomed. Opt. 13 (2008) 054057.
[63] W.A. Baumgartner, et al., Blood flow switching among pulmonary capillaries is decreased during high hematocrit, J. Appl. Physiol. 97 (2004) 522–526.
[64] M. Yanai, J.P. Butler, T. Suzuki, A. Kanda, M. Kurachi, H. Tashiro, H. Sasaki, Intracellular elasticity and viscosity in the body, leading, and trailing regions of locomoting neutrophils, Am. J. Physiol. Cell Physiol. 277 (1999) C432–C440.
[65] E.R. Weibel, Morphometry of the Human Lungs, Springer-Verlag, Berlin, 1963.
[66] H. Spencer, Morphometry of the human lung, J. Anat. 98 (1964) 457.
[67] G.W. Schmid-Schönbein, Y.Y. Shih, S. Chien, Morphometry of human leukocytes, Blood 56 (1980) 866–875.
[68] S. Chien, Role of blood cells in microcirculatory regulation, Microvasc. Res. 29 (1985) 129–151.
[69] J.C. Hogg, Neutrophil kinetics and lung injury, Physiol. Rev. 67 (1987) 1249–1295.
[70] F. Moazzam, F.A. DeLano, B.W. Zweifach, G.W. Schmid-Schönbein, The leukocyte response to fluid stress, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 5338–5343.
[71] A.E. Ekpenyong, et al., Viscoelastic properties of differentiating blood cells are fate- and function-dependent, PLoS One 7 (2012) e45237.

[72] T. Lämmermann, et al., Rapid leukocyte migration by integrin-independent flowing and squeezing, Nature 453 (2008) 51–55.
[73] B. Yap, R.D. Kamm, Cytoskeletal remodeling and cellular activation during deformation of neutrophils into narrow channels, J. Appl. Physiol. 99 (2005) 2323–2330.
[74] B. Tabor, et al., Dialysis neutropenia: the role of the cytoskeleton, Kidney Int. 53 (1998) 783–789.
[75] M.R. Looney, et al., Stabilized imaging of immune surveillance in the mouse lung, Nat. Methods 8 (2011) 91–96.
[76] R. Tran-Son-Tay, D. Needham, A. Yeung, R.M. Hochmuth, Time-dependent recovery of passive neutrophils after large deformation, Biophys. J. 60 (1991) 856–866.
[77] C. Summers, et al., Establishing the pulmonary transit time of primed and unprimed neutrophils in man, Crit. Care Med. (2010) 2790.
[78] P. Roca-Cusachs, et al., Rheology of passive and adhesion-activated neutrophils probed by atomic force microscopy, Biophys. J. 91 (2006) 3508–3518.
[79] E.M. Craciun, F. Altfelder, N. Kuss, J. Poeschl, P. Ruef, Anti-inflammatory effects of selected drugs on activated neonatal and adult neutrophils, Scand. J. Clin. Lab. Investig. 73 (2013) 407–413.
[80] E.J. Kawaoka, M.E. Miller, A.T. Cheung, Chemotactic factor-induced effects upon deformability of human polymorphonuclear leukocytes, J. Clin. Immunol. 1 (1981) 41–44.
[81] D.R. Gossett, et al., Hydrodynamic stretching of single cells for large population mechanical phenotyping, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 7630–7635.
[82] H.T.K. Tse, et al., Quantitative diagnosis of malignant pleural effusions by single-cell mechanophenotyping, Sci. Transl. Med. 5 (2013) 212ra163.
[83] W. Pan, et al., Viscoelastic properties of human mesenchymal stem cells, IEEE Eng Med Biol Soc 5 (2005) 4854–4857.
[84] R.D. González-Cruz, V.C. Fonseca, E.M. Darling, Cellular mechanical properties reflect the differentiation potential of adipose-derived mesenchymal stem cells, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 1523–1529.
[85] P. Kollmannsberger, C.T. Mierke, B. Fabry, Nonlinear viscoelasticity of adherent cells is controlled by cytoskeletal tension, Soft Matter 7 (2011) 3127.
[86] O. Thoumine, A. Ott, Time scale dependent viscoelastic and contractile regimes in fibroblasts probed by microplate manipulation, J. Cell Sci. 110 (1997) 2109–2116.
[87] M.J. Rosenbluth, W.A. Lam, D.A. Fletcher, Force microscopy of nonadherent cells: a comparison of leukemia cell deformability, Biophys. J. 90 (2006) 2994–3003.
[88] J.M. Dealy, Official nomenclature for material functions describing the response of a viscoelastic fluid to various shearing and extensional deformations, J. Rheol. 39 (1995) 253–265.
[89] J. Guck, R. Ananthakrishnan, T.J. Moon, C.C. Cunningham, J. Käs, Optical deformability of soft biological dielectrics, Phys. Rev. Lett. 84 (2000) 5451–5454.
[90] J. Guck, et al., The optical stretcher: a novel laser tool to micromanipulate cells, Biophys. J. 81 (2001) 767–784.
[91] P. Hinterdorfer, C. Le Grimellec, D. Navajas, P. Parot, Y.F. Dufre, Past, present and future of atomic force microscopy in life sciences and medicine, J. Mol. Recognit. 20 (2007) 418–431.
[92] T.G. Mason, K. Ganesan, J.H. van Zanten, D. Wirtz, S.C. Kuo, Particle tracking microrheology of complex fluids, Phys. Rev. Lett. 79 (1997) 3282–3285.
[93] L. Buzanska, et al., Patterned growth and differentiation of human cord blood-derived neural stem cells on bio-functionalized surfaces, Acta Neurobiol. Exp. (Wars) 69 (2009) 24–36.
[94] M. Kondo, et al., Biology of hematopoietic stem cells and progenitors: implications for clinical application, Annu. Rev. Immunol. 21 (2003) 759–806.
[95] H. Saito, Mechanical properties of rat bone marrow and circulating neutrophils and their responses to inflammatory mediators, Blood 99 (2002) 2207–2213.
[96] Y. Komai, G.W. Schmid-Schönbein, De-activation of neutrophils in suspension by fluid shear stress: a requirement for erythrocytes, Ann. Biomed. Eng. 33 (2005) 1375–1386.
[97] F. Moazzam, F.A. DeLano, B.W. Zweifach, G.W. Schmid-Schönbein, The leukocyte response to fluid stress, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 5338–5343.
[98] S. Fukuda, T. Yasu, D.N. Predescu, G.W. Schmid-scho, Mechanisms for regulation of fluid shear stress response in circulating leukocytes, Circ. Res. (2000) 1–6.
[99] P. Marschel, G.W. Schmid-Schönbein, Control of fluid shear response in circulating leukocytes by integrins, Ann. Biomed. Eng. 30 (2002) 333–343.
[100] S. Fukuda, G.W. Schmid-Schönbein, Regulation of CD18 expression on neutrophils in response to fluid shear stress, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13152–13157.
[101] S. Fukuda, G.W. Schmid-Schönbein, Centrifugation attenuates the fluid shear response of circulating leukocytes, 72 (2002) 133–139.
[102] S. Fukuda, H. Mitsuoka, G.W. Schmid-Schönbein, Leukocyte fluid shear response in the presence of glucocorticoid, J. Leukoc. Biol. 75 (2004) 664–670.
[103] N.R. Singh, C. Summers, A. Johnston, A.M. Peters, E.R. Chilvers, Differential Effects of Sepsis and Acute Respiratory Distress Syndrome (ARDS) on Cd62l Expression in Neutrophils Entering and Leaving the Lung, Am. J. Respir. Crit. Care Med. 185 (2012) A1037.
[104] C. Summers, et al., Pulmonary retention of primed neutrophils: a novel protective host response, which is impaired in the acute respiratory distress syndrome, Thorax 69 (2014) 623–629.
[105] Y. Zhao, S. Chien, S. Weinbaum, Dynamic contact forces on leukocyte microvilli and their penetration of the endothelial glycocalyx, Biophys. J. 80 (2001) 1124–1140.
[106] W.M. Nauseef, N. Borregaard, Neutrophils at work, Nat. Immunol. 15 (2014) 602–611.
[107] K.M. Stroka, H. Aranda-espinoza, Endothelial cell substrate stiffness influences neutrophil transmigration via myosin light chain kinase-dependent cell contraction, Blood 118 (2014) 1632–1641.
[108] A. Rabodzey, P. Alcaide, F.W. Luscinskas, B. Ladoux, Mechanical forces induced by the transendothelial migration of human neutrophils, Biophys. J. 95 (2008) 1428–1438.
[109] A. Mócsai, Diverse novel functions of neutrophils in immunity, inflammation, and beyond, J. Exp. Med. 210 (2013) 1283–1299.
[110] D.E. Discher, P. Janmey, Y.-L. Wang, Tissue cells feel and respond to the stiffness of their substrate, Science 310 (2005) 1139–1143 (80-).
[111] P.W. Oakes, et al., Neutrophil morphology and migration are affected by substrate elasticity, Blood 114 (2009) 1387–1395.
[112] T. Yeung, et al., Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion, Cell Motil. Cytoskeleton 60 (2005) 24–34.
[113] R.A. Jannat, M. Dembo, D.A. Hammer, Traction forces of neutrophils migrating on compliant substrates, Biophys. J. 101 (2011) 575–584.
[114] W.J. Polacheck, A.E. German, A. Mammoto, D.E. Ingber, R.D. Kamm, Mechanotransduction of fluid stresses governs 3D cell migration, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 2447–2452.
[115] A.L. Robertson, et al., A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism, Sci. Transl. Med. 6 (2014) 225ra29.
[116] S.M. Man, et al., Actin polymerization as a key innate immune effector mechanism to control *Salmonella* infection, Proc. Natl. Acad. Sci. U. S. A. 111 (2014).
[117] P. Sundd, et al., "Slings" enable neutrophil rolling at high shear, Nature 488 (2012) 399–403.
[118] K. Yoshida, R. Kondo, Q. Wang, C.M. Doerschuk, Neutrophil cytoskeletal rearrangements during capillary sequestration in bacterial pneumonia in rats, Am. J. Respir. Crit. Care Med. 174 (2006) 689–698.
[119] A.T. Skoutelis, et al., Effect of cytokines and colony-stimulating factors on passive polymorphonuclear leukocyte deformability in vitro, Cytokine 12 (2000) 1737–1740.
[120] M.L. Gardel, et al., Stress-dependent elasticity of composite actin networks as a model for cell behavior, Phys. Rev. Lett. 96 (2006) 088102.
[121] J. Stricker, T. Falzone, M.L. Gardel, Mechanics of the F-actin cytoskeleton, J. Biomech. 43 (2010) 9–14.
[122] C. Haslett, L.A. Guthrie, M.M. Kopaniak, R.B. Johnston, P.M. Henson, Modulation of multiple neutrophil functions by preparative methods or trace concentrations of bacterial lipopolysaccharide, Am. J. Pathol. 119 (1985) 101–110.
[123] P.J. Krause, et al., Modulation of neonatal neutrophil function by pentoxifylline, Pediatr. Res. 29 (1991) 123–127.
[124] J. Pillay, et al., In vivo labeling with $2H_2O$ reveals a human neutrophil lifespan of 5.4 days, Blood 116 (2010) 625–627.
[125] C. Summers, E.R. Chilvers, A.M. Peters, Mathematical modeling supports the presence of neutrophil depriming in vivo, Physiol. Rep. 2 (2014) e00241.
[126] E.B. Finger, et al., Adhesion through L-selectin requires a threshold hydrodynamic shear, Nature 379 (1996) 266–269.
[127] M.F. Coughlin, G.W. Schmid-Schönbein, Pseudopod projection and cell spreading of passive leukocytes in response to fluid shear stress, Biophys. J. 87 (2004) 2035–2042.
[128] A. Makino, et al., G protein-coupled receptors serve as mechanosensors for fluid shear stress in neutrophils, Am. J. Physiol. Cell Physiol. 290 (2006) C1633–C1639.
[129] J.D. Humphrey, E.R. Dufresne, M.A. Schwartz, Mechanotransduction and extracellular matrix homeostasis, Nat. Rev. Mol. Cell Biol. 15 (2014) 802–812.
[130] M.J. Mitchell, M.R. King, Shear-induced resistance to neutrophil activation via the formyl peptide receptor, Biophys. J. 102 (2012) 1804–1814.
[131] M.J. Mitchell, K.S. Lin, M.R. King, Fluid shear stress increases neutrophil activation via platelet-activating factor, Biophys. J. 106 (2014) 2243–2253.
[132] X. Zhang, D. Zhan, H.Y. Shin, Integrin subtype-dependent CD18 cleavage under shear and its influence on leukocyte-platelet binding, J. Leukoc. Biol. 93 (2013) 251–258.
[133] X. Zhang, D. Zhan, H.Y. Shin, Leukocyte mechanosensitivity to fluid shear stress depends on the subtypes of CD18 integrins, FASEB J. 26 (2012) 680.7.
[134] S. Kruss, et al., Adhesion maturation of neutrophils on nanoscopically presented platelet glycoprotein Ibα, ACS Nano 7 (2013) 9984–9996.
[135] Z.T. Han, et al., 12-O-Tetradecanoylphorbol-13-acetate (TPA)-induced increase in depressed white blood cell counts in patients treated with cytotoxic cancer chemotherapeutic drugs, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 5362–5365.
[136] C. Weber, H. Noels, Atherosclerosis: current pathogenesis and therapeutic options, Nat. Med. 17 (2011) 1410–1422.
[137] M. Naegele, et al., Neutrophils in multiple sclerosis are characterized by a primed phenotype, J. Neuroimmunol. 242 (2012) 60–71.
[138] E.M. Drost, W. Macnee, S.F. Smith, The effect of lipocortin 1 on neutrophil deformability, Mediators Inflamm. 5 (1996) 188–190.
[139] F.S. Lakschevitz, M.B. Visser, C. Sun, M. Glogauer, Neutrophil transcriptional profile changes during transit from bone marrow to sites of inflammation, Cell. Mol. Immunol. 12 (2015) 53–65.