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# Microfluidics to Mimic Blood Flow in Health and Disease

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## Keywords

microfluidics, lab-on-a-chip technology, shear forces, blood flow, red blood cells, cardiovascular models

## Abstract

Throughout history, capillary systems have aided the establishment of the fundamental laws of blood flow and its non-Newtonian properties. The advent of microfluidics technology in the 1990s propelled the development of highly integrated lab-on-a-chip platforms that allow highly accurate replication of vascular systems' dimensions, mechanical properties, and biological complexity. Applications include the detection of pathological changes to red blood cells, white blood cells, and platelets at unparalleled sensitivity and the efficacy assessment of drug treatment. Recent efforts have aimed at the development of microfluidics-based tests usable in a clinial environment or the replication of more complex diseases such as thrombosis. These microfluidic disease models enable the study of onset and progression of disease as well as the identification of key players and risk factors, which have led to a spectrum of clinically relevant findings.

## 1. INTRODUCTION

**Lateral migration:** shear-induced cell/vesicle movement toward the channel center, accompanied by tank treading, shape deformation, and constant inclination

**Fåhraeus effect:** red blood cell (RBC) concentration scales with blood vessel diameter (between 10 and 300 μm) due to lateral migration of RBCs away from the vessel wall; the Fåhraeus effect influences the Fåhraeus-Lindqvist effect

**Fåhraeus–Lindqvist effect:** apparent blood viscosity scales with blood vessel diameter (vessel diameters between 10 and 300 μm) due to lateral migration of red blood cells away from the vessel wall

Humans, animals, and plants employ microchannels to transport fluids, cells, and molecules over longer distances. For instance, an adult human has different types of tissues for fluid transport, e.g., the vascular system, with larger arteries and veins and smaller capillaries, and the lymphatic system, with a less well-defined liquid drainage system. These transport systems are great in extent: The combined length of blood vessels in an adult human sums up to approximately 100,000 km, with diameters of typically 5 μm to 4 mm, and are constantly transporting 5–6 L of blood through the body, reaching every remote tissue. The entire vascular system is thereby exposed to mechanical forces such as gravity and stretch, strain, and shear forces. Blood vessels, endothelial cells (ECs), blood cells, and other components are all flexible and deformable. Despite the considerable magnitude of pressure and shear forces present in the blood vessels and capillary system (**Table 1**), the exposed cells withstand these forces—in fact, they are perfectly adjusted to them. Any smaller lesions can be tolerated or even healed by the body; however, constant dysfunction may lead to severe diseases and irreparable defects (Ross & Glomset 1976).

For a long time, researchers have aimed to understand the cardiovascular system on all levels, i.e., the nature and properties of the vessels, as well as the complex behavior of blood and its components flowing through the human body. Although, historically, much effort focused on determining the laws of fluid mechanics inside blood vessels with simple equipment, today we have advanced technology—e.g., microfluidic instruments, biochemical technology, and high-tech microscopy—to mimic natural systems with more and more precision and to obtain insights into cellular and molecular key players in these complex systems.

The growing complexity of microfluidic systems has brought increasingly realistic models that enhance our understanding of the emergence and progression of cardiovascular function, disease, and treatment. Moreover, research and modeling of blood systems provide insights into many other relevant phenomena, e.g., the transport and attachment of biomarkers, signaling factors, exosomes, and cells (including circulating tumor cells all over the body), as well as the distribution of molecules and drugs in the body. Cardiovascular models may reveal important aspects of tissue engineering or can give insights into design principles for biomimetic construction of transport systems. **Figure 1** summarizes schematically the fundamental effects of shear forces on the dynamics of cells and their use in microfluidic applications. The governing flow types (**Figure 1*a*,*b***) lead to the characteristic behavior of individual red blood cells (RBCs), such as the lateral migration towards the channel center in flow channels or in blood vessels with diameters greater than 30 μm (**Figure 1*c***) or the compressive forces in narrow capillaries, a prerequisite for optimal gas exchange between RBCs and the surrounding tissue (**Figure 1*d***). These single cell dynamics lead to the characteristic flow phenomena of blood, most importantly the Fåhraeus–Lindqvist effect (**Figure 1*e***). Microfluidic technology allows researchers to reconstitute the flow

**Table 1 Shear values and diameters of blood vessels in the cardiovascular system**

| Name | Shear rate ($s^{-1}$) | Shear stress ($dyn/cm^2$) | Vessel diameter (mm) |
|---|---|---|---|
| Capillaries | 1,300 | 43 | 0.005–0.010 |
| Arterioles | 1,600 | 58 | 0.100–0.300 |
| Small artery | 1,350 | 49 | 0.300–0.500 |
| Coronary artery | 450 | 15 | 3.5 |
| Femoral artery | 300 | 10 | 5.0 |
| Ascending aorta | 200 | 5 | 33.0 |

Data adapted from Papaioannou et al. (2005) and Sakariassen et al. (2015).

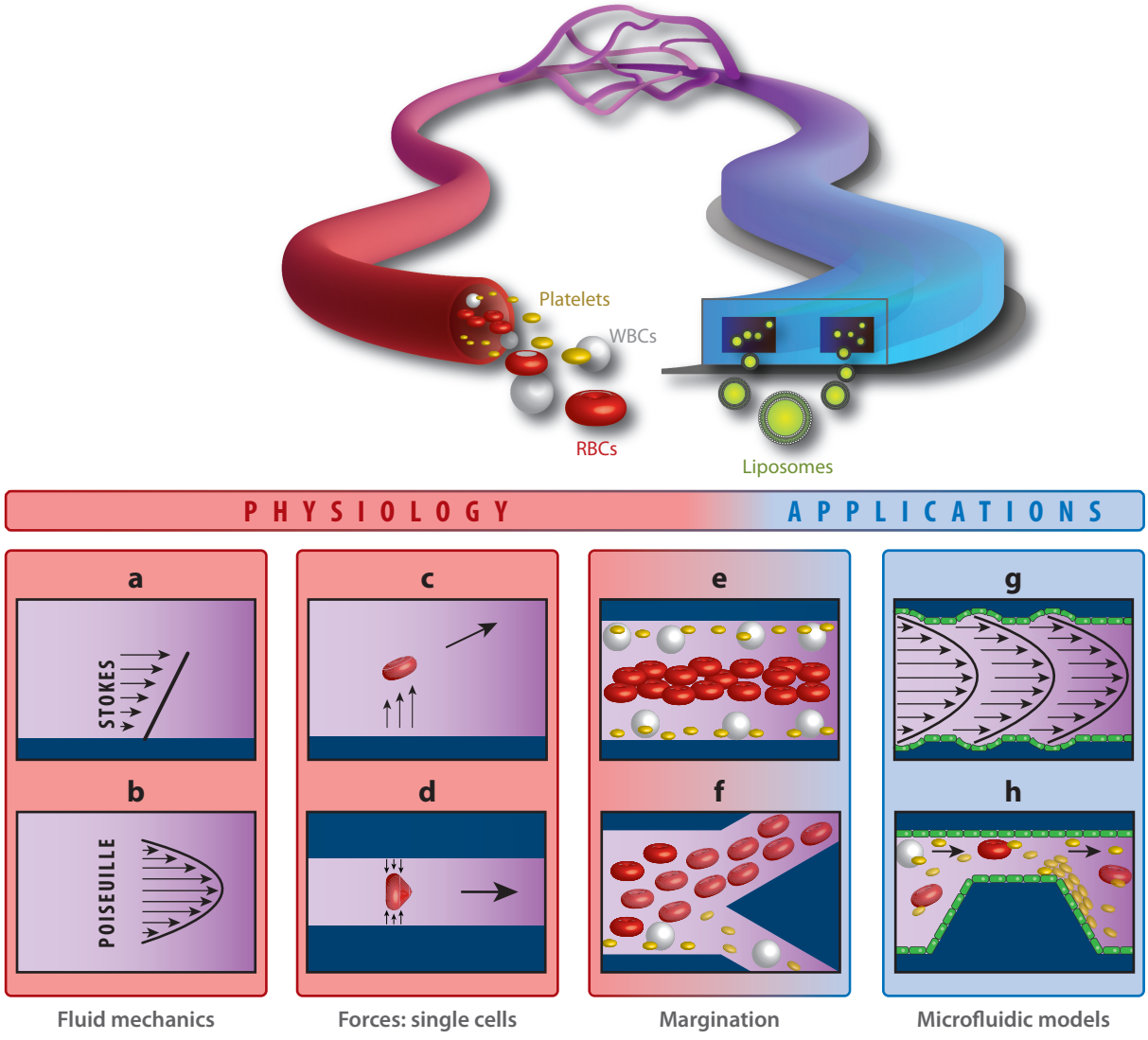


**Figure 1**

From physiology to technology: the cardiovascular system and its microfluidic models. Blood flow through microvessels is of viscous nature, resulting in (*a*) Stokes flow near the walls and (*b*) the characteristic Poiseuille flow away from the walls. Shear forces result from these phenomena that act on cells depending on their shape, deformability, and size. Shear forces and cell–wall interactions cause red blood cells (RBCs) inside blood vessels with diameters >30 μm to deform and (*c*) finally migrate toward the channel center (Fåhraeus–Lindqvist effect). (*d*) Inside blood vessels with cell-sized diameters or smaller, cell–wall interactions compress RBCs, leading to optimal gas exchange with the surrounding tissue. Their high abundance causes RBCs to dominate hemodynamics as well as white blood cell (WBC) and platelet (PLT) dynamics. (*e*) This leads to the characteristic near-wall depletion of RBCs and the margination of WBCs and PLTs. Microfluidic models specifically use these hemodynamic properties in applications such as the filtration of WBCs (*f*) or analysis of RBCs with pathologically altered deformability. Microfluidic models may replicate physiological and pathological conditions at high accuracy by use of biomimetic surfaces composed of vascular cells and associated proteins, pulsed flow (*g*), and geometric constraints. This enables the study of complex interactions occurring throughout disease onset and progression such as cardiovascular disease (*h*).

**Hemorheology:** describes the flow properties of blood and the dynamics of its constituents, cells and plasma

phenomena of single cells and cell suspensions in controlled, artificial environments and has brought many applications employing these phenomena to the study of the cardiovascular system in health and disease. Applications include the filtration of malaria-infected or healthy RBCs from blood (**Figure 1*f***), studies of the effect of pulsed flows on ECs (**Figure 1*g***), and the reconstitution of complex processes like wound healing or cardiovascular disease (**Figure 1*h***).

In this review, we discuss shear-mediated blood flow phenomena and their application in microfluidic systems. We initially discuss microfluidic technology and recent approaches to replicate blood vessels by microfabrication. After introducing the fundamental dynamics of RBCs, white blood cells (WBCs), and platelets (PLTs), we review microdevices for mimicking physiological and pathological blood flow. Theoretical work on these topics is not covered.

## 2. MICROFLUIDIC TECHNOLOGY

Microfluidic systems can be designed with diameters varying roughly from the nanometer scale up to a few hundred micrometers, and with lengths up to several centimeters. Depending on the fabrication technique and substrate choice, channels with rectangular or round cross sections can be built, scaffolding can be introduced, and the rigidity can be tailored (Unger et al. 2000, Whitesides 2006, Xia & Whitesides 1998). The commonly used substrates are polymers, among them polydimethylsiloxane (PDMS). PDMS is gas permeable and therefore enables cell culturing inside microfluidic channels constructed from this material. Appropriate surface patterns or cell-adhesive or -repellent coatings enable locally defined cell adhesion on microfluidic channels made from PDMS (Zhao et al. 1997). In addition to modifying the surface chemistry, flow conditions can be varied.

Microfluidic systems have been extensively employed for biomechanical studies in recent years (Kurth et al. 2012). Many studies address the effects of flow and shear stress on cells, e.g., the activation of specific receptors and ion channels (Kurth et al. 2015), their influence on cell development (Toh & Voldman 2011), and changes in the cytoskeleton (Birukov et al. 2002). Microfluidic platforms are ideally suited to apply other forces on target objects such as stretch and compression (Huh et al. 2010). Moreover, microsystem technology allows small sensors to measure the forces exerted by the cells. An example is the fabrication of flexible microposts to observe the effects of shear force exerted by adherent cells cultured on top (Lam et al. 2012). Shear-stressed cells exert forces on the micropost array while aligning and elongating in the direction of flow. These cellular forces can be quantified by measuring micropost deflection.

In recent years, microfluidic platforms have been designed not only to capture, culture, and analyze cells, but also to precisely mimic different types of tissues, including lung (Huh et al. 2007) and heart (Giridharan et al. 2010, McCain et al. 2014). These so-called organs-on-a-chip play a vital role in new drug discovery and drug testing strategies (Skommer & Wlodkowic 2015, Sung & Shuler 2009). Several studies report on the combination of different tissue types that are hosted on a single platform to study the metabolic pathway of a drug molecule (Sung & Shuler 2009) or the co-culture of different cell types (Mi et al. 2016). This trend emphasizes the potential of microfluidic platforms for understanding the role of specific body functions. However, it is also important for organs-on-a-chip devices to precisely mimic the cardiovasculature.

## 3. BLOOD VESSELS AND THEIR MICROFLUIDIC MODELS

Cardiovascular models are important for the investigation of hemorheology and the deduction of the governing physical laws. The law of Hagen–Poiseuille, dating back to 1840, laid the foundation of fluid mechanics and hemorheology from observations of water inside a glass tube (Sutera & Skalak 1993). Glass capillaries of defined dimensions are simple vascular models from a modern-day perspective, yet fully account for several fundamental discoveries, such as shear

thinning in small capillaries, known as the Fåhraeus–Lindqvist effect (Fåhraeus & Lindqvist 1931), or shear thickening in even smaller capillaries (Dintenfass 1967).

For a more realistic model of the natural vessels, the geometries, branching structure, substrate material, and surfaces must be more thoroughly designed. Cokelet et al. (1993) were among the first to fabricate microvascular blood vessel networks by micro systems technology from semicircular (rigid) glass plates containing bifurcated channels. Changes in the design were later made with respect to the geometry and cross section (Fiddes et al. 2010), as well as the angles and the specific design of bifurcations (Barber & Emerson 2007, Lee & Lee 2009). About 90 years ago, Murray (1926) pointed out that the cube of the diameter of the parent vessels equals the sum of the cubes of the diameters of the daughter vessels, known as Murray's law. Lim et al. (2003) demonstrated that a chip design compliant with Murray's law provides stable and homogeneous flow velocities in all channels. They also highlighted the importance of changing channel heights according to Murray's law. In other words, the standard fabrication by photolithography that provides only one uniform height is not optimal. Alternatively, a technique based on direct laser writing allows the creation of channels of different heights on a single platform. Rosano et al. (2009) have introduced an interesting way of mimicking in vivo vascular networks. They took in vivo images (stained with fluorescent fluorescein isothiocyanate–dextran) and copied the bifurcations, tortuosities, and cross-sectional changes into a PDMS-made microchip to culture ECs over several days. However, none of the abovementioned model systems accounted for the flexibility of the blood vessels and their surface properties.

Tomaiuolo et al. (2011) investigated the effect of microchannel material on RBC deformability. Cell deformation was less pronounced in PDMS microchannels than in glass capillaries. Due to the lower Young's modulus of PDMS compared to glass, cell-wall interactions cause local deformations in the channel walls, whereas glass capillaries of similar diameter remain unchanged (**Figure 2*a***). In addition, polymer brushes adhered to the walls of microcapillaries serve as a first approximation of a model of the supramolecular layer (glycocalyx) lining the inside of blood vessels (Bureau et al. 2017). In their presence, an increase in flow resistance similar to in vivo observations was measured inside 10-μm capillaries. RBC deformation was found to increase with brush thickness in contrast to lateral velocity, which remained unaffected.

Other approaches to surface functionalization are the use of cultured ECs, realized by use of perfusion systems and growth chambers. Song et al. (2009) reported on approaches in this direction and recreated physiological conditions for the ECs, thereby emphasizing the importance of the natural shear strength levels and the application of pulsatile flow, as ECs can discriminate between pulsatile and constant flow (**Figure 1*g***). This was confirmed by Estrada et al. (2011), who additionally accounted for the flexibility of the blood vessel walls by the addition of a thin and flexible PDMS membrane. Under physiological conditions of pressure, flow, and stretch and shear stress, human aortic ECs exhibited in vivo–like phenotypes with respect to size, alignment of the cytoskeletal actin filament, and the expression levels of the marker β-catenin. In other approaches, Bischel et al. (2013) have presented a simple method to pattern three-dimensional (3D) lumen structures through hydrogels that can be lined with ECs to mimic 3D vessel structures in a variety of microchannel geometries. Y. Zheng et al. (2012a) formed endothelialized microfluidic vessels within a native collagen matrix. The abovementioned simple planar systems with linear or branched channel networks are robust and are used in many applied studies for blood flow analysis, as discussed in the subsequent sections. They highlight the importance of the careful choice of experimental flow conditions, as summarized in **Table 1**.

More sophisticated microdevices demonstrate the future potential for mimicking complex processes or diseases that involve the vascular system. Wang and coworkers (2016) reported a microfluidic network that allowed for rebuilding various stages of the vascular development,

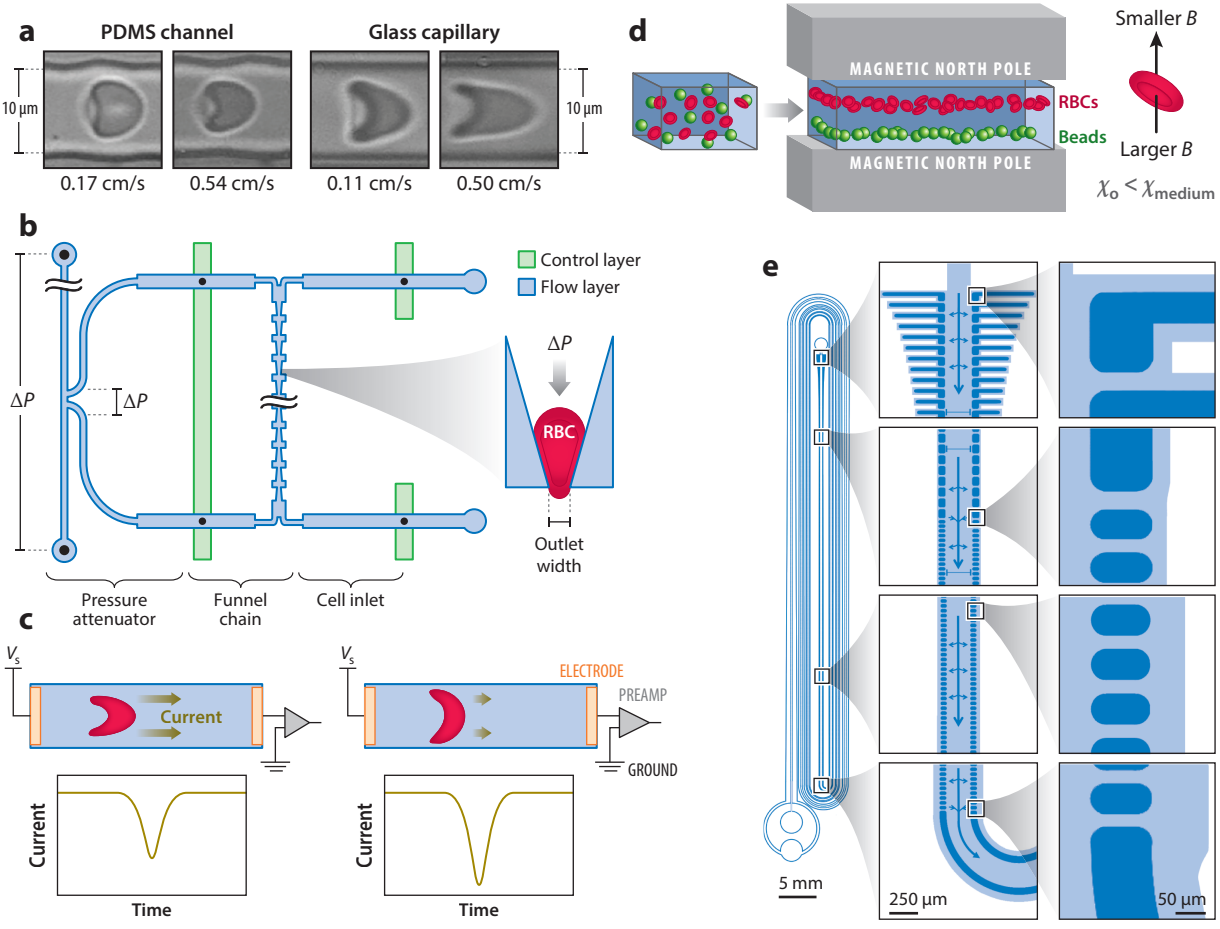


**Figure 2**

Microfluidic platforms for deformability-based RBC studies find a wide variety of applications, from fundamental research to the studies of diseases. (*a*) RBC deformation in PDMS channels and glass capillaries (Tomaiuolo et al. 2011). Adapted with permission from the Royal Society of Chemistry. (*b*) RBCs pass through microchannel constrictions at defined pressures in a deformability-dependent manner. The microfluidic device enables application of finely tuned pressures using an on-chip attenuation system, thereby detecting malaria-infected RBCs at various developmental stages of the parasite (Guo et al. 2012, 2014). Adapted with permission from the Royal Society of Chemistry. (*c*) The deformation of RBCs inside narrow capillaries was measured using electrical opacity (Zheng et al. 2013). Adapted with permission from John Wiley & Sons. (*d*) Simultaneous cell sorting and density-based analysis is possible using a magnetic medium. This method can be used to detect pathological changes in RBC density and has been readily implemented in a point-of-care microfluidic platform (Tasoglu et al. 2015). (*e*) Controlled incremental filtration allows for continuous, passive sorting of WBCs by taking advantage of their margination dynamics (Xia et al. 2016). Abbreviations: B, magnetic field strength; RBC, red blood cell; PDMS, polydimethylsiloxane; WBC, white blood cell; $\chi$, magnetic susceptibility.

including vasculogenesis, EC lining, sprouting angiogenesis, and anastomosis. In their device design, side channels serve as arteries and veins and are connected with central tissue chambers (Wang et al. 2016). Such vascular model systems mimic the fluid circulation in vasculature-rich tissues (X. Li et al. 2014, Mi et al. 2016) and are a further step toward organs-on-a-chip. Other work addresses the process of wound healing (Han et al. 2012) and the regeneration of vasculature.

In addition to the replication of the vasculature for blood flow investigations, model systems are highly relevant for investigating the effects of shear forces and cyclic stretch on ECs (Rossi

et al. 2009, W. Zheng et al. 2012). For example, the flow rates in a microdevice can be varied in small steps, and the resulting shear stress can be simulated for the given channel geometries. Rossi et al. (2009) continuously varied the shear strength applied to an EC on a single chip by use of a tapered channel. In this way, all other conditions are constant and the comparison of cells at different positions is possible. In addition, the researchers used particle imaging velocimetry to determine the flow speed precisely within the channel and to profile the surface topography of the EC layer. Moreover, further parameters can be easily varied, such as the supply of nutrients. For example, Yu et al. (2013) investigated EC apoptosis under hyperglycemia conditions present in diabetes patients.

In conclusion, the emergence of microsystem technology in the 1990s today facilitates the mimicking of sophisticated artificial cardiovascular systems. The basic dimensions of a blood vessel, i.e., diameters of 5 μm to 4 mm and the typical lengths of 500 μm to several centimeters, can be conveniently realized in engineered microchannel systems by use of optical photolithography. However, precisely reconstituting the conditions of arteries and veins, the biomechanical parameters, and the interactions with cells and tissues in the body remains a persistent challenge.

## 4. RED BLOOD CELLS

Blood is a suspension of erythrocytes, WBCs, and PLTs in a Newtonian liquid, the plasma. RBCs are the most abundant cell type in blood (~99%) and constitute up to 45% of the blood volume (hematocrit) in humans. RBC dynamics primarily determine whole blood rheology with immediate effects on WBC and PLT dynamics.

### 4.1. Red Blood Cell Dynamics

In the blood flow, RBC shape and dynamics primarily depend on the magnitude of shear forces across blood vessels. As shear forces scale with vessel diameter, different flow phenomena can be observed in large blood vessels and microcapillaries. The shear-dependent dynamics of single cells and RBCs in blood are discussed in this section. They are fundamental to the microfluidic platforms discussed in the following sections.

**4.1.1. Single cell dynamics.** The Fåhraeus–Lindqvist effect constitutes the dominant shear force–mediated flow phenomenon of RBC suspensions in blood vessels of 30–300 μm in diameter. It is caused by lift forces acting on individual RBCs and cell–cell interactions. RBCs initially moving near the vessel walls consequently migrate laterally toward the channel center (**Figure 1*c***). Much has been learned about the fundamental dynamics by studying lipid vesicles as cell models (Abkarian & Viallat 2005, 2008; Abkarian et al. 2002; Callens et al. 2008; Coupier et al. 2008; Lorz et al. 2000). Positively buoyant vesicles initially move along the channel bottom and then lift off and gradually move toward the channel center, where they assume an equilibrium position. This leads to an increased concentration of vesicles in the channel center compared to near-wall regions. Vesicles experience different forces in the vicinity of the channel walls than in the channel center. Near the walls, Stokes (creeping) flow and strong vesicle–wall interactions dominate, termed wall-bounded flow (Abkarian & Viallat 2005). Vesicles deform into prolate ellipsoids and tilt upwards so that their long axis points toward the channel center with increasing shear, and finally lift off (Abkarian et al. 2002, Callens et al. 2008, Lorz et al. 2000). These dynamics are accompanied by a rotation of the vesicle membrane around its center of mass, termed tank treading, which stabilizes its orientation. At some distance to the channel bottom, wall effects cease and nonlinear shear dominates vesicle dynamics, termed unbounded flow. Vesicle deformation and lift velocity decrease toward the channel center, and the vesicle eventually assumes an equilibrium position.

Lift velocity depends on the viscosity and osmotic ratio between inner and outer fluids, as well as the shear rate.

Similar dynamics have been observed for RBCs, although quantitative experimental studies on the lateral migration of individual cells are rare in contrast to vesicles (Abkarian & Viallat 2008, Geislinger et al. 2012). Lateral migration of RBCs, however, coincides with the transition between two different shear-dependent dynamic states: solid-like to fluid-like (Dupire et al. 2012). During solid-like motion, the entire cell tumbles in a head-over-heels manner with oscillating orientation, known as tumbling. Above a critical shear rate, a transition to fluid-like dynamics is observed, characterized by tank-treading motion. The transition to tank treading, however, is a prerequisite for the onset of lateral migration. Cell deformability plays an important role for the onset of tank treading. Chemically stiffened cells are less deformable and exhibit a delayed tumbling-to-tank-treading transition toward higher shear rates. Consequently, the onset of lateral migration is delayed toward higher shear rates as well (Dupire et al. 2012; Forsyth et al. 2010, 2011). These findings imply that different dynamics are found in RBCs with pathologically modified deformability. Researchers using microfluidic devices to investigate diseases that affect RBC deformability focus on the detection of these subtle changes in cell dynamics (see Section 4.3).

**Tank treading:** rotational movement of a cell or vesicle membrane around its lumen; this movement stabilizes the cell/vesicle orientation

**Tumbling:** rotational, solid-like movement of the entire cell or vesicle, reminiscent of a coin-flipping movement with unsteady orbit angle

**Margination:** movement toward the channel walls; in blood, white blood cells and platelets marginate as a consequence of red blood cell dynamics

**4.1.2. Red blood cell suspensions.** Starting from individual RBCs, the investigation of their dynamics in cell suspensions is the first step toward understanding blood rheology. In suspensions, RBCs interact through cell–cell collisions or hydrodynamic interaction. It is known from vesicle studies (Kantsler et al. 2008) that hydrodynamic interactions cause severe changes in shape and velocity that can impact lateral migration. Similar studies on the suspension properties of RBCs find that cell–cell interactions counterbalance lateral migration of individual cells and cause a lift flux in the suspension (Grandchamp et al. 2013). The resulting RBC distribution shows the characteristic near-wall depletion and increased cell concentration in the channel center. Due to the high abundance of RBCs in blood, RBC dynamics substantially dominate hemorheology as well as the dynamics of less abundant blood cells (see Section 5.1).

**4.1.3. Implications for white blood cells and platelets.** Constant interactions of WBCs and PLTs with the far more abundant RBCs force these cells toward the blood vessel walls, a process called margination (**Figure 1*e***) (Goldsmith & Spain 1984, Nobis et al. 1985, Schmid-Schönbein et al. 1980). Evidence for the importance of cell–cell interactions for WBC and PLT margination was found by studying particles inside microfluidic channels. Spherical particles, unsusceptible to lift forces in purely viscous flows (Goldsmith & Mason 1961), distribute homogenously across the channel cross section in shear flows due to particle–particle interactions. In suspensions of RBCs and particles, however, they marginate (D'Apolito et al. 2016) in a size-dependent manner (Charoenphol et al. 2010, Zhao et al. 2007). The low particle deformability compared to RBCs further supports the margination behavior of WBCs. PLT margination is more efficient compared to WBCs, due to their lenticular shape and smaller size. RBC suspensions spiked with PLT-shaped particles showed shear-dependent cross-stream diffusion in contrast to spherical particles (Rusconi & Stone 2008).

## 4.2. Microfluidic Platforms Investigating Shear-Induced Deformation in Red Blood Cells

The great influence of RBC deformability on hemorheology renders this property a potential measure of homeostasis and marker of disease (Chien 1987). In the past, RBC deformability has been measured using micropipette aspiration, optical trapping, and other techniques such as

laser diffraction viscometry (ektocytometry) (Clark et al. 2016, Linderkamp & Meiselman 1982, Rancourt-Grenier et al. 2010). Microfluidic methods enable different direct and indirect measurements of deformability at high throughputs and sensitivity.

RBCs flowing through glass capillaries experience transient shape evolution depending on the RBC characteristic shape recovery time, which can serve as marker of RBC deformability (**Figure 1*d***) (Tomaiuolo & Guido 2011). Continuing development of this platform brought forth a microfluidic platform for the quantitative analysis of multiple cell parameters such as RBC recovery time and RBC shape, which allows identification and analysis of pathological RBCs (Tomaiuolo et al. 2016). Guo et al. (2014) developed a microfluidics analog of the pipette aspiration method. Cells were guided to an analysis channel consisting of a series of narrowing funnels using on-chip valves, and RBCs were subsequently trapped (**Figure 2*b***). The driving pressure was increased, resulting in increasing pushing forces on the trapped RBC, which eventually squeezed through the funnel at a threshold pressure. RBC cortical tension was calculated on a single-cell level with respect to the critical pressure, funnel geometry, and cell size. Deformability deviations of RBCs stiffened with glutaraldehyde could be detected for applied concentrations as low as 0.001%, which demonstrates the high sensitivity of the RBC deformability measurement of this microfluidic platform. The device positively detected oxygenated RBCs in patient-derived blood samples. This single-use, low-cost microfluidic platform could be used in clinically relevant settings such as suitability analysis of stored blood for transfusion (Kwan et al. 2013).

An electrical measurement of cell deformability made use of the shear effects on single RBCs (Zheng et al. 2013). Cells flow through microchannels with dimensions similar to the cell size at constant shear. Cell–wall interactions cause less rigid RBCs to deform more, which results in a greater distance between the cell membrane and the channel walls. The severity of deformation scales with the signal strength of an electric current applied along the microfluidic channel (see **Figure 2*c***). Cell deformability can be deduced from the signal strength via the characteristic elongation index of healthy RBCs. The measurement principle allows for throughput up to 150 cells per second while screening cell biophysical properties (Y. Zheng et al. 2012b).

A new approach measures the levitation of RBCs in a magnetic medium, commonly applied as a contrast agent in magnetic resonance imaging, to assess cell deformability (**Figure 2*d***). RBCs are immersed in a gadolinium solution and injected into a microcapillary sandwiched between two rare earth magnets (Felton et al. 2016, Tasoglu et al. 2015). The magnetic force decreases vertically from the capillary bottom to the top, and the resulting equilibrium position of the RBC is therefore defined by the cell density. This method allows for differentiation between anemic and healthy RBCs (Felton et al. 2016) and could find further use in passive sorting of blood cells. The medical potential for the detection of diseases such as malaria, iron deficiency, or sickle cell disease (SCD) inspired the authors to develop a smartphone-based setup.

## 4.3. Microfluidic Platforms Investigating Pathologically Impaired Red Blood Cell Deformability

Abnormal RBC deformability changes rheological blood properties, which makes it a potential marker of disease. It has been associated with diabetes (Carroll et al. 2006), malaria (Dondorp et al. 1997, 2000), SCD (Epstein & Bunn 1997), and other diseases.

**4.3.1. Malaria.** Dramatic changes in RBC deformability are found in malaria, where parasites invade and thereby infect RBCs. During the erythrocytic cycle, a parasite infects an RBC, grows by digesting intracellular hemoglobin (trophozoite stage), and proliferates (schizont stage) until the host cell is so crowded that it breaks open and releases young parasites into the bloodstream, which now can infect more RBCs. The maturing parasite excretes proteins to the cell surface,

rendering it adhesive while its deformability decreases progressively. Toward the end of parasite maturation, infected RBCs (iRBCs) may marginate and sequester on the vascular endothelium, causing capillary blockage, possibly resulting in organ failure (Dondorp et al. 2000, Handayani et al. 2009, Shelby et al. 2003). iRBC deformability and shape recovery have been identified as primary indicators of severe malaria in humans (Dondorp et al. 1997, 1999).

Throughout parasite maturation, iRBCs deformability decreases 1.5 to 200 times compared to noninfected cells (Guo et al. 2012). Reduced deformability causes iRBCs to be less affected by lift forces compared to healthy RBCs. iRBCs therefore marginate in suspensions containing both healthy and infected RBCs, as is the case in the blood of malaria patients. Several studies made use of iRBC margination for microfluidic cell separation through side channels from healthy RBCs in the channel center (Geislinger et al. 2014, Hou et al. 2010). On-chip separation through margination efficiently removes iRBCs from samples, allowing the use of whole (unprocessed) blood rather than time-consuming sample preprocessing (Bow et al. 2011). Analysis of collected RBCs showed 75–90% capture efficiency of iRBCs in the side channels (Hou et al. 2010). Detection of early-stage malaria is generally difficult to achieve due to low parasitemia levels, but could increase chances of survival (Dondorp et al. 1997, Kong et al. 2015). A carefully optimized microfluidic platform comprising parallel margination channels increased the concentration of early–trophozoite stage iRBCs through the separation of marginated iRBCs, followed by an off-chip magnetic resonance relaxometry analysis. Parasitemia levels down to 0.0005% could be detected through highly efficient sample preconcentration and thus at earlier stages of disease progression than could be achieved using ektacytometry (Kong et al. 2015). Furthermore, filters can be easily implemented in microfluidic device designs that efficiently separate RBCs from blood, allowing the use of whole blood for disease diagnosis, thereby eliminating the need for preprocessing patient blood. Due to their impaired deformability and shape relaxation, iRBCs possess higher fluidic resistance. Their travel time through channels featuring periodic constrictions is therefore greater than for healthy RBCs. Arrays of parallelized analysis channels allow large amounts of RBCs to be processed and for ring stage (early trophozoite) iRBCs to be detected in blood with low parasitemia levels (Bow et al. 2011). Detection of early-stage malaria using this approach may significantly decrease the case fatality rate.

Other microfluidic approaches measure deformability by squeezing RBCs through microcapillaries with diameters smaller than the cell size (Shelby et al. 2003). Healthy RBCs and ring stage iRBCs passed through constrictions of 8-, 6-, 4-, and 2-μm diameters. Trophozoite iRBCs did not pass through 2- and 4-μm constrictions, whereas late-stage iRBCs (schizonts) also failed to pass through 6-μm constrictions and rapidly formed capillary blockages. Shape recovery was delayed in ring stage iRBCs after passing through 4-μm constrictions, whereas schizonts did not recover at all. This approach could be used for passive sorting of iRBCs with respect to parasite maturation. Similar studies of *Plasmodium vivax*, however, showed high iRBC deformability compared to *Plasmodium falciparum*. Infected cells readily passed through 2-μm-wide constrictions, whereas 15% of healthy RBCs were destroyed, which makes this parasite much more difficult to detect or filter by deformability-based methods (Handayani et al. 2009).

**4.3.2. Sickle cell disease.** A genetic deficiency on the oxygen carrier hemoglobin causes severe shape deformations (sickling) of RBCs with reduced oxygenation (hypoxia) (Epstein & Bunn 1997). Besides cell sickling, affected RBCs show decreased deformability and increased internal cell viscosity. RBC deoxygenation in the capillary system triggers sickle hemoglobin polymerization followed by RBC sickling within seconds. Vaso-occlusion in the capillary system is a frequent consequence of cell sickling in SCD (Epstein & Bunn 1997).

Microfluidic platforms focus on the investigation of the modified cell density and deformability of sickle RBCs with regard to their hypoxia level. Chip designs aim at on-demand induction of

hypoxia in RBCs to study the effects of cell sickling. Such platforms take advantage of the gas permeability of PDMS, which allows perfusion of blood samples with oxygen during analysis. Studies target the effect of vaso-occlusion (Higgins et al. 2007, Wood et al. 2012), adhesion to model blood vessels functionalized with bioactive coatings (Alapan et al. 2016b, 2014; Kim et al. 2016; Little et al. 2014), and deformation of RBCs (Alapan et al. 2014). Microfluidic devices typically consist of two vertically stacked PDMS layers. The bottom layer consists of analysis channels through which blood samples are flushed. A thin PDMS membrane separates the flow layer from the gas perfusion layer on top, allowing precise control of oxygen levels in samples (**Figure 3*a***) (Higgins et al. 2007, Wood et al. 2012). With these platforms, oxygen-dependent polymerization of sickle hemoglobin could be triggered, leading to vaso-occlusion and rescue. The study revealed critical channel diameters, oxygen concentrations, and driving pressures necessary for the occurrence of occlusion and rescue (Higgins et al. 2007). Furthermore, deoxygenation-induced cell sickling led to a decrease in blood flow despite constant driving pressure and allowed for a velocity-based determination of the severity of the disease (Wood et al. 2012).

Alapan et al. (2014, 2016a,b) studied adhesion and detachment of sickled red cells on fibrin-coated PDMS surfaces exposed to laminar flow. Microfluidic platforms mimic postcapillary venules in size and surface properties due to a coating with endothelium-associated proteins. Whole blood is flushed in and sickle RBCs adhere to the artificial capillaries at physiological flow rates. Results show higher sensitivity to physiological changes in the flow rate (Little et al. 2014). Adhesion-based studies could furthermore quantify different adhesion levels among hemoglobin phenotypes (Alapan et al. 2016a). Moreover, Alapan et al. (2014, 2016b) have integrated deformability and adhesion measurements into a single microfluidic platform. Two subspecies of sickled cells were discovered, either deformable or nondeformable, with respect to their aspect ratio in flow and during detachment (Alapan et al. 2014). Bimodal response to hypoxia was supported by Kim et al. (2016). Such microfluidic platforms show great potential in clinical use, as they combine sensitive and cost-effective diagnoses of disease severity, have very low sample consumption, and promote adhesion as a new clinical biomarker.

Other microfluidic platforms assess the efficacy of existing treatments. Hydroxyurea is the only available FDA-approved drug, although there are conflicting reports on its effects (Li et al. 2015, Rollins et al. 2015, Tsai et al. 2012). Interaction studies between blood and model vasculatures have produced astonishing findings of PLT-mediated vaso-occlusive risk for SCD. Increased shear-independent PLT aggregation was found in blood samples from SCD patients compared to healthy controls and could be reduced by treatment with hydroxyurea (Rollins et al. 2015).

PDMS-based microfluidic chips have been used to investigate rheological properties of sickle blood. On-chip induction of hypoxia in sickle RBCs and subsequent analysis showed improvement or worsening of blood rheology with respect to hypoxia duration, yet failed to associate blood viscosity with severity of SCD.

The geographic distribution of SCD casualties in sub-Saharan regions and the Arabic peninsula demands that diagnoses be made using highly autonomous platforms, because clinical-grade health facilities may be unavailable. Recently, a paper-based point-of-care device for hemoglobin separation using cellulose acetate electrophoresis was successfully validated against standard clinical methods (Ung et al. 2015). The low-cost, single-use device works autonomously using finger prick blood. Web-based image processing analyses and diagnoses are obtained using a mobile phone.

## 5. WHITE BLOOD CELLS

WBCs are immune cells that circulate in the vascular and lymphatic systems, accounting for 1% of blood volume. In their inactive state, WBCs possess a nearly spherical shape of 10–12 μm

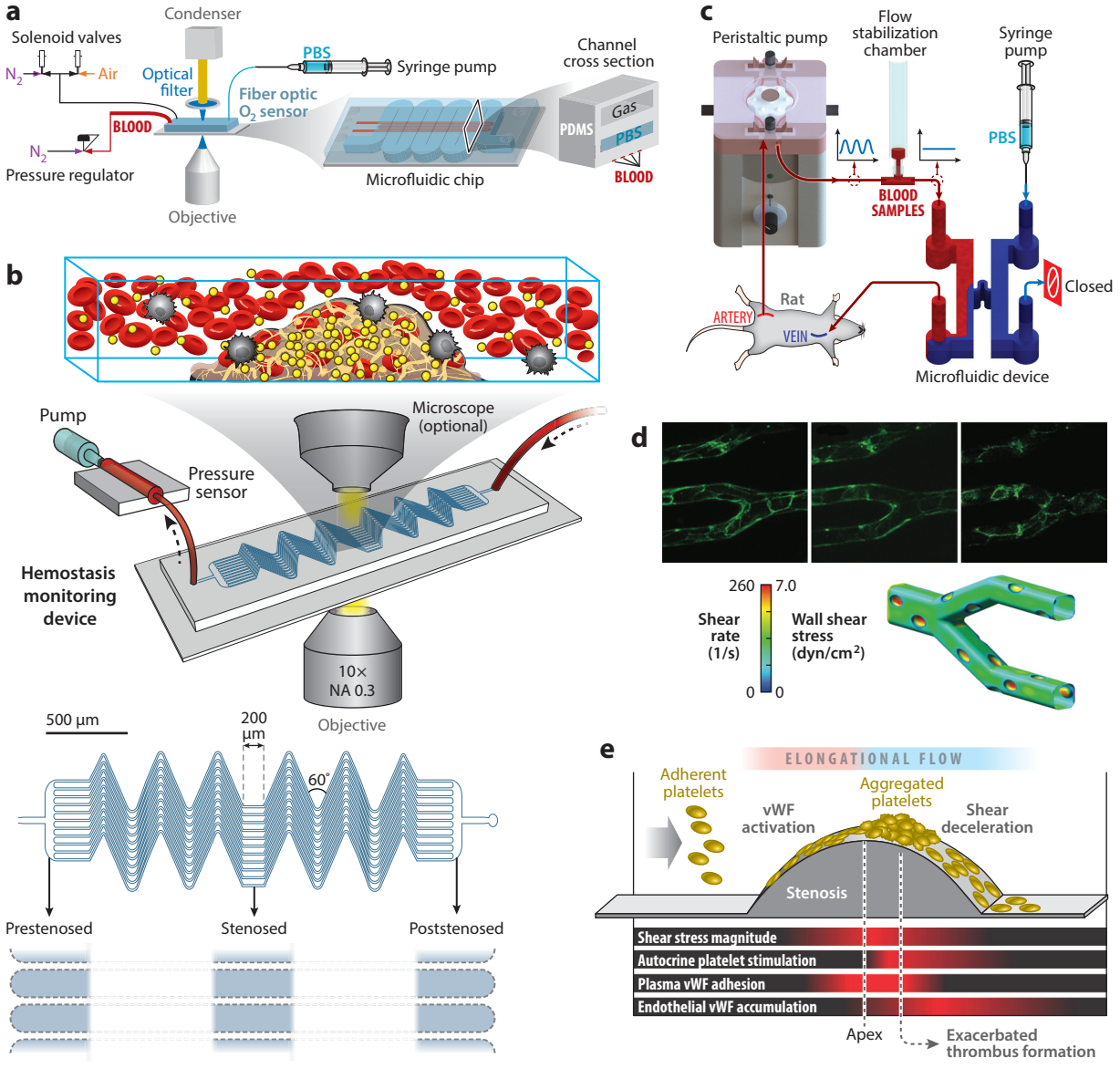


**Figure 3**

Advanced microfluidic platforms model physiological and pathophysiological aspects of the cardiovascular system. (*a*) The combination of a blood flow layer and a gas perfusion layer allows for control of RBC sickling under defined hypoxia conditions (Wood et al. 2012). (*b*) A microfluidic channel system mimics stenosed arteriolar blood vessels. The platform has been successfully used for real-time surveillance during anticoagulation therapy (Jain et al. 2016). (*c*) Ex vivo surveillance of hemostasis using an extracorporeal microfluidic model (Yeom et al. 2016). (*d*) Microfluidic vascular model for the study of vaso-occlusive risk in sickle cell disease revealed a shear dependence of this risk (Tsai et al. 2012). (*e*) The shear dependence of thrombus formation and growth can be studied in constrictions inside microfluidic channels (Westein et al. 2013). Abbreviations: PBS, phosphate-buffered saline; PDMS, polydimethylsiloxane; RBC, red blood cell; vWF, won Willebrand factor.

in diameter for the most abundant WBC types. WBCs play a vital role in the human immune system. WBC dynamics consist of margination, rolling, migration, and extravasation. This multistep process is tightly regulated biochemically and mechanically by means of shear stress. Failure throughout this process has been associated with the onset of various diseases such as multiple sclerosis, atherosclerosis, and asthma (Bianchi et al. 2013).

## 5.1. Microfluidic Platforms for the Investigation of White Blood Cell Dynamics

WBCs show shear-dependent dynamics besides margination. They exit the blood flow by adhesion to the vascular endothelium. Arrest and extravasation of WBCs are preceded by shear-dependent rolling (Atherton & Born 1973), mediated by transient protein interactions between WBCs and the endothelium (Kansas 1996, Lawrence & Springer 1993). During rolling, the endothelium communicates potential inflammation to the WBC through chemokines on its surface. Eventually, WBCs form pseudopods in a shear-dependent manner (Moazzam et al. 1997) and extravasate from the cardiovascular system by transmigration through the vascular endothelium.

The characteristic WBC and PLT margination dynamics can be used to filter WBCs out of blood transfusions, which reduces multiple transfusion-related complications (Heal & Blumberg 2004). Controlled incremental filtration (CIF) is a passive filtration technique that enables highly efficient upconcentration of marginated WBCs and PLTs from blood (**Figure 1*f***). In CIF, a main channel is flanked by two parallel side channels with regularly distributed connections in between, through which marginated WBCs and PLTs are collected (**Figure 2*e***). Flow simulation was used to drive the design toward highly controlled flows in the microchannels (Gifford et al. 2014, Xia et al. 2016). Increased throughput and less risk of clogging are advantageous features of this technique for handling patient blood samples. With the CIF device, WBC concentration was reduced 1,000-fold from PLT-rich plasma (Xia et al. 2016). The interplay of computer simulations and microfabrication is essential for the development of microfluidic platforms driven toward precisely adjusted flows and high throughput.

During inflammatory response, WBCs exit the blood flow, adhering to and rolling on the vascular endothelium, and finally exit the blood vessel. WBCs in contact with the extracellular matrix show shear-dependent dynamics governed by the passing blood. Proteins on the surface of the vascular endothelium mediate WBC recruitment from blood. Microfluidic platforms for the investigation of WBC adhesion thus employ surface functionalization using adhesion-promoting proteins (Watts et al. 2013) or EC monolayers cultured inside the microchannels (Chau et al. 2009, Schaff et al. 2007).

Cell adhesion and detachment studies have led to different microfluidic device designs based on the experimental time frame. Long-term experiments require a constant perfusion of on-chip cell cultures. A microchannel network design featuring different channel heights allows for simultaneous reagent supply and shear flow experiments. Short experiments do not necessarily require feeding the cells with medium and can be realized with simpler microchannel designs of a single height (Lu et al. 2004). On-chip cell cultures are generally difficult to realize and are time consuming, facts that motivated the development of simpler and more straightforward on-chip cell culturing strategies. Hybrid designs that interface well plates with microfluidic platforms may overcome this difficulty (Conant et al. 2011, Schaff et al. 2007). Schaff et al. (2007) characterized the shear- and chemokine-dependent kinetics of WBC rolling in such a hybrid device.

Other studies of WBC dynamics investigate their chemotactic behavior. Microfluidic platforms allow cell capture in defined locations and perfusion with chemical gradients (Saadi et al. 2007) as well as rapid switching between chemicals. More elaborate designs integrate spatial and temporal variability of chemical gradients. Irimia et al. (2006) used on-chip membrane valves for precise

flow and chemical gradient control in their two-level chip design. They observed that WBCs, initially migrating toward chemokine gradients, could be depolarized through a change in gradient orientation. The use of hydrogels in chemotaxis studies is an interesting alternative to PDMS due to its higher permeability. In agarose microfluidic chips, chemical gradients are established between two separated parallel channels (Cheng et al. 2007). Transmigration studies that rely on microfabricated devices are rare in the literature and leave room for microfluidics applications in the future. One example is the microfluidic Boyden chamber of Schreiber et al. (2007) used to study chemically induced transmigration through a porous filter.

### 5.2. Microfluidic Platforms Investigating Impaired White Blood Cell Dynamics

Fay et al. (2016) evaluated the effect of anti-inflammatory drugs such as dexamethasone on WBC adhesion. Their combinatorial approach compared different processes governing margination and adhesion of WBCs in microcapillaries and veins. Different microfluidic platforms replicated the cross-sectional dimensions of the respective blood vessel type and were perfused with blood at physiological flow rates. Reduction of cellular stiffness through cytoskeletal remodeling was identified as a new drug-mediated mechanism leading to demargination of WBCs in both blood vessel models. The absence of standard tests for in vitro investigation of drug efficacy and biophysical effects attribute great clinical relevance to such microfluidic platforms.

Tsai et al. (2012) proposed a microvasculature-on-a-chip model that integrates multiple biophysical cues. The geometry and dimensions of a microvascular arterial network are replicated on a microfluidic device. Microchannel surfaces are functionalized with ECs, thereby recreating mechanical and biochemical properties of the EC matrix (**Figure 3*b***). WBCs were perfused through the microchannels adhered to the model vasculature at physiological rates. The use of an adhesion-promoting signaling protein caused EC activation and led to an increase in WBC adhesion and consequent microchannel obstruction. Additional WBC activation caused these cells to stiffen, which modified their dynamic properties. The reduced deformability of activated cells resulted in more efficient margination. The combination of increased WBC margination and EC adhesiveness caused a dramatic increase in channel blockage. The nonlinear effects of combined EC and WBC activation on blood vessel obstruction clearly demonstrate the need for and importance of complex, physiological WBC adhesion models that integrate geometrical, rheological, and biological aspects of blood vessels. The versatility and clinical relevance of this microvasculature model were demonstrated in studies evaluating the efficacy of drugs in SCD patients' blood (Tsai et al. 2012).

## 6. PLATELETS

PLTs, or thrombocytes, are anuclear blood cells with a biconcave discoid shape in their inactive state, measuring 2–4 μm in diameter and 0.5 μm in thickness. Although more abundant than WBCs, they constitute merely ∼0.1% of the blood volume due to their small size. PLTs play an important role in wound healing; however, their influence on hemorheology is negligible. In their activated state, chemical signaling leads to plug formation and the induction of primary coagulation.

### 6.1. Platelet Function and Dynamics

Wound healing is initiated by the accumulation of PLTs at the injury site. At physiological shear, PLTs bind directly to the exposed collagen or via the glycoprotein von Willebrand factor

(vWF) (Bergmeier et al. 2006, Denis et al. 1998, Jackson et al. 2009, Nuyttens et al. 2010). PLT adhesion onto immobilized vWF is independent of PLT activation at pathological shear rates above 400 dyn/cm$^2$, yet unstable. Above 800 dyn/cm$^2$, adherent PLTs become stretched and nucleate stable clots (Ruggeri 2006). Furthermore, hematocrit has a positive influence on shear-assisted PLT adhesion onto immobilized vWF (Chen et al. 2013). PLT aggregation, however, is primarily governed by blood rheology rather than biochemical interactions, and thrombus formation preferentially occurs in low-shear zones on the downstream side of initial aggregates (Nesbitt et al. 2009).

## 6.2. Microfluidic Platforms Investigating Platelet Function and Dynamics Related to Cardiovascular Disease

Thrombosis is the most frequent cause of common cardiovascular diseases such as ischemic heart disease, stroke, and venous thromboembolism, which together account for more than 25% of all disease-related deaths worldwide (Int. Soc. Thromb. Haemost. Steer. Comm. World Thromb. Day 2014). Microfluidic platforms may mimic capillary constriction (stenosis) of increasing obstruction, whereas channel surfaces can be functionalized with complex extracellular matrix models composed of proteins and/or cells in a straightforward manner. Functionalization of PDMS microchannels with adhesion promoters involved in wound healing, like vWF, has been systematically investigated (Conant et al. 2011, Gutierrez et al. 2008, Hansen et al. 2011). PLT adhesion assays could discriminate von Willebrand disease (vWD) blood samples from controls by means of PLT adhesion dynamics (Hansen et al. 2011). Advanced platforms integrate hundreds of lesion models into a single device resulting in highly quantitative investigations (Hansen et al. 2012). PLT adhesion and thrombus growth at different shear rates in vitro reenact different stages of the disease (Conant et al. 2011, Kantak et al. 2003, Kent et al. 2010, Shi et al. 2016). PLT adhesion was promoted by vWF at pathological shear and resulted in accelerated thrombus growth (Conant et al. 2011, Kent et al. 2010, Shi et al. 2016, Song et al. 2013). Direct observations revealed thrombi of different shapes and structures at physiological and pathophysiological shear (Shi et al. 2016). The latter type easily sheds from its growth site and blocks downstream blood vessels, creating great risk of tissue damage or stroke.

PLT aggregation studies have led to the development of more complex microfluidic platforms that reproduce stenosis together with functionalized surfaces in order to study the shear-mediated progression of cardiovascular diseases (Jain et al. 2016, Li et al. 2012, Para & Ku 2013, Westein et al. 2013). Tovar-Lopez et al. (2010) systematically investigated the effect of microshear gradients generated by vascular constrictions on PLT aggregation in vitro. Different constriction geometries were designed ranging from gradual to abrupt (immediate constriction), thereby generating a variety of shear gradients. Findings support the role of geometric properties of vessel constrictions, which establish distinct shear flows, in aggregate formation and stabilization (Tovar-Lopez et al. 2010). However, the dynamics and mechanisms of PLT aggregation around stenosed blood vessels remain poorly understood. Shear-dependent PLT adhesion onto flow-modulating geometries precoated with vWF at physiological and pathological flows could show clinically relevant diagnosis of the disease (Nandurkar et al. 2016).

The development of stenosis models for the use of clinical samples under physiological and pathological flows generated more sensitive readouts than standard clinical tests. Jain et al. (2016) replicated stenosis in arterioles using a bifurcating channel system consisting of accelerating (prestenotic), constant (stenotic), and decelerating (poststenotic) sections (**Figure 3*c***). The approach uses clotting time as a marker of hemostasis levels in patient blood. Clotting time was shear-dependent at pathological flow rates, similar to clotting times in patients with bleeding

disorders. Ex vivo coagulation monitoring of a living pig using this device showed great potential for real-time patient surveillance in a clinical setting (Jain et al. 2016). Other ex vivo studies allow constant monitoring of biophysical markers of cardiovascular disease in blood, thereby eliminating the need for lab-based evaluation of collected blood samples (**Figure 3*d***) (Yeom et al. 2016). Westein et al. (2013) replicated the geometric and rheological properties of stenosis previously investigated in a mouse (**Figure 3*e***). The microfluidic model featured semicircular constrictions blocking between 20% and 80% of the microchannels in order to account for various sizes of atherosclerotic plaques. Westein et al. (2013) used flow simulation to optimize the device design in order to accurately mimic shear rates of stenotic and nonstenotic regions in vivo. The experimental design replicated in vivo stenosis at various levels of biological complexity, ranging from whole blood perfusion to interaction networks between patterned EC monolayers, adhesion promoters (vWF, fibrinogen), and inhibitors (iloprost) with PLTs. The study revealed the location of forming thrombi in the stenotic outlet regions. Upon plaque rupture, thrombi are likely to form at the injury site and precipitate cardiovascular disease. This identified blood-borne shear-sensitive vWF as a key player and mediator of PLT aggregation (Westein et al. 2013).

Furthermore, efficacy testing of existing therapeutic approaches has been carried out using microfluidic thrombosis models. Shear-dependent clot formation from PLTs has been investigated under therapeutic concentration of anticoagulant drugs (Jain et al. 2016, M. Li et al. 2014, Para & Ku 2013, Tsai et al. 2012, Yeom et al. 2016). Due to highly quantitative measurements and reduced complexity of the vascular models compared to in vivo studies, these studies may contribute to a more accurate understanding of vascular diseases and therapeutics.

Microfluidic platforms for the study of platelet dynamics have helped unravel the fundamental processes of and biochemical interactions during wound healing and cardiovascular disease. In particular, research in this area has benefitted from the use of microfabrication technologies to pattern microfluidic channel surfaces with cells and proteins of the vascular endothelium and to mimic the flow conditions of cardiovascular disease at various stages.

## 7. SUMMARY, CONCLUSIONS, AND FUTURE ISSUES

Throughout history, increasingly complex models of blood and blood vessels have created a fundamental understanding of hemorheology and blood cells. The advent of microfluidics technology enabled the design of microfluidic platforms that precisely mimic blood vessels. Computational flow simulation furthermore improved device designs to accurately replicate in vivo flow dynamics. Functionalization of microchannel surfaces with ECs and associated proteins enables replication of complex diseases such as thrombosis. Additionally, these platforms serve very well as sample preparation and analysis tools, for which they exploit the margination behavior of WBCs, PLTs, or RBCs with impaired deformability for cell filtration, increased concentration, and downstream analysis.

Microfluidic platforms mainly assess the pathophysiological behavior of RBCs by measuring RBC deformability, a disease marker and indicator of severity in malaria, SCD, and others. Alternative detection methods of impaired RBC deformability, such as magnetic levitation, electrical opacity, or shape relaxation, have emerged in recent years. Besides diagnostics, RBC deformability platforms are applied for efficacy assessment of pharmacological treatment. The ongoing development of mechanical RBC characterization may result in new tools for clinical application and point-of-care diagnostics and identify new disease markers in the near future.

WBCs and PLTs are involved in complex interactions of immune response and cardiovascular and other diseases. Microfluidic platforms investigating WBC and PLT dynamics and functions range from simple laminar flow assays to complex model vasculatures comprising on-chip cell

cultures and channel geometries that reconstitute blood flows of cardiovascular disease. Efficacy testing or monitoring of drug-based therapeutics are promising medical applications of microfluidic devices.

Combined integration of engineered tissues, biophysical interaction networks, and mechanical properties into microfluidic devices will lead to microfluidic models of rising physiological accuracy in the future. The growing complexity of such models will further attribute more clinical significance to their findings and may ultimately establish microfluidic vascular models in clinical applications.

## DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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