

Microcirculatory blood flow in health and disease

by

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A dissertation submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy
(Chemical and Biological Engineering)

at the

UNIVERSITY OF WISCONSIN - MADISON

2025

Date of final oral examination: Feb 11, 2025

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To my beloved family.

Dedication

To my family:

I am profoundly grateful to my parents and my sister for their unwavering support, encouragement, and love throughout this journey. Their steadfast belief in me has been an endless source of strength and motivation, empowering me to overcome challenges and strive for excellence.

To my advisor, committee, and research collaborators:

I wish to express my heartfelt gratitude to my PhD advisor, Prof. Michael Graham, for his invaluable guidance, mentorship, and enduring patience over the years. Your profound insights and unwavering encouragement have been instrumental in shaping my academic growth and enabling the completion of this thesis. I am also deeply thankful to my committee members, Prof. Join Yin, Prof. Reid Van Lehn, and Prof. Saverio Spagnolie, for their constructive feedback, thoughtful support, and meaningful contributions to my research. I would also like to thank my research collaborators, Prof. Wilbur Lam, Christina Caruso, and Elizabeth Iffrig, for their invaluable contributions and insights. Furthermore, I am indebted to the many professors at UW-Madison whose classes and mentorship have enriched my educational journey. Their dedication and expertise have provided me with a robust foundation and a wealth of knowledge.

To my friends:

I am immensely thankful to the past and present members of our research group for their collaboration, camaraderie, and support. A special acknowledgment goes to Alec

Linot, Kevin Zeng, Eric Yu, Daniel Floryan, Carlos Pérez De Jesús, RJ Hommel, Ricardo Constante-Amores, Ashwin Shekar, Alex Guo, Charlie Young, Andrew Fox, Jake Buzhardt, Manish Kumar, Dell Zimmerman, Hayden Boline, and Ashinth Mangesh. Your friendship and shared moments have significantly enriched my journey and made this experience both fulfilling and memorable.

I would also like to extend my sincere gratitude to my friends at Madison for their unwavering support and encouragement: Jiaze Ma, Panzheng Zhou, Hui Geng, Jiayang Wu, Wei Lin, Jie Zhou, Yisen Wang, Yifu Chen, Yaqing Wu, and my cohorts. Your kindness and companionship have been invaluable to me, and I am truly grateful for the moments we have shared along the way.

Abstract

Blood flow at the cellular level in the microcirculation plays a crucial role in both healthy physiological processes and pathological conditions. However, this complex phenomenon remains poorly understood. This dissertation investigates the dynamics of cellular-level blood flow in microcirculation, with a focus on the distribution and collective behavior of blood's cellular components. Particular attention is given to interactions between cells and vessel walls, especially within complex vascular geometries. The research examines how cellular segregation and margination influence hemodynamics, with an emphasis on blood disorders characterized by altered physical properties of cells and intercellular interactions, such as increased red blood cell (RBC) aggregation. Using computational modeling validated by experimental data, the study provides insights into how abnormal RBC properties and aggregation contribute to vascular stress fluctuations, endothelial damage, and glycocalyx disruption. In addition, the investigation examines cellular partitioning at vascular bifurcations, uncovering mechanisms that drive the progression of vascular damage in conditions like sickle cell disease within vascular networks. The robustness of the computational framework is established through verification and validation, enabling accurate simulations of blood flow in various vascular geometries. Together, these findings provide a foundation for understanding microvascular biomechanics and offer valuable directions for future research on vascular dysfunction in blood disorders.

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General introduction

1.1 Blood Components and Physiological Functions

Blood is a confined, flowing suspension of various cellular species within an aqueous medium called plasma [9]. It comprises several principal components: red blood cells (RBCs, or erythrocytes), white blood cells (WBCs, or leukocytes), platelets (PLTs), and plasma, each fulfilling distinct physiological roles. Blood, as a complex biofluid, exhibits complexity in both microstructural and mechanical senses—as a multiphase, non-Newtonian, viscoelastic fluid—and in a biological sense, functioning as a tissue with diverse roles. These roles include the transport of oxygen and nutrients, as well as the coordination of responses to injury and inflammation. RBCs are the predominant cellular component of blood. The volume fraction of RBCs, known as hematocrit, ranges from 10% to 30% in the microcirculation and 40% to 50% in larger vessels such as arteries. RBCs vastly outnumber WBCs and PLTs, with ratios of approximately 500–1000:1 and 12–32:1, respectively [10].

The physical properties of RBCs, WBCs, and PLTs—such as their shape, size, and stiffness—vary significantly. A mature RBC is a biconcave discoid about 8 μm across and 2 μm high [11]. Such a unique shape facilitates mass transport across the cell membrane due to a high surface-to-volume ratio. Neutrophils, the majority of WBCs, take an almost spherical shape about 10–12 μm across [12]. Moreover, PLTs are 1–2 μm cell fragments and discoidal under normal conditions. RBCs are extremely deformable particles, whereas WBCs and PLTs are much stiffer. It has no nucleus or cytoskeleton and to a first approximation can be viewed mechanically as a deformable fluid-filled capsule with an elastic membrane [11]. Extreme deformation of RBCs allows them to squeeze through narrow microvessels with dimensions even smaller than their size. The primary function of RBCs is oxygen transport with the intracellular oxygen-carrying protein, hemoglobin. WBCs are part of the immune system and are actively involved in defense against infection caused by bacteria, or foreign bodies [13]. Formed in bone marrow via pinching off from megakaryocytes, PLTs play a crucial role in the coagulation process, the formation of thrombosis, and atherosclerosis [14, 15]. These are central elements of cardiovascular and cerebrovascular diseases[16].

Plasma, constituting approximately 55% of the total blood volume, serves as the medium for transporting all cellular components, along with a vast array of small molecules and proteins. Composed primarily of water, plasma is generally regarded as an incompressible and Newtonian fluid. Plasma plays a vital role in maintaining physiological function. Dissolved within plasma are numerous small molecules, including gases, salts, urea, hormones, amino acids, lipids, glucose, and vitamins, as well as a variety of proteins [17]. Albumin, a compact and globular protein, not only acts as a carrier for many small molecules but also significantly contributes to the osmotic pressure of blood. Globulins perform diverse roles such as the transport of other proteins and participation in immune responses. Many proteins in plasma play critical roles in the coagulation process [14, 18]. Fibrinogen, a key protein, polymerizes into fibrin during coagulation, forming the primary structural component of blood clots [19]. Additionally, fibrin contributes to the stabilization of platelet aggregates following vascular injury [20]. Von Willebrand factor (VWF) is another essential protein involved in coagulation. It is found both in plasma and within the endothelial cells lining blood vessels. VWF exists in various sizes, with its fully extended forms reaching lengths of approximately $100\text{ }\mu\text{m}$ [21]. This protein plays a crucial role in clot formation by facilitating platelet adhesion and aggregation under specific conditions [22–24].

1.2 Microvessels, Endothelium, and Glycocalyx Layer

Microvessels, the small blood vessels of the circulatory system, include capillaries, arterioles, and venules [25]. Microvessels are vital for facilitating the exchange of oxygen, nutrients, and waste products between the blood and the interstitial fluid. Capillaries, the smallest microvessels, range in diameter from $5\text{ }\mu\text{m}$ to $10\text{ }\mu\text{m}$ and are composed solely of the tunica intima, a thin layer of simple squamous endothelial cells [26]. This minimal structural design optimizes capillaries for their primary function of enabling efficient molecular exchange with the surrounding interstitial fluid, essential for cellular function and tissue health. Arterioles

play a crucial role in regulating blood flow into capillary networks through vasoconstriction and vasodilation, mediated by the contraction and relaxation of smooth muscle cells [27]. These vessels possess muscular walls and are the primary site of vascular resistance. The transition from arterioles to capillaries is marked by significant changes in blood pressure and flow velocity, emphasizing their importance in microvascular hemodynamics. Venules, on the other hand, function to collect blood from capillaries and transport it toward larger veins. Ranging in diameter from $10\ \mu\text{m}$ to $30\ \mu\text{m}$, venules serve as intermediaries between the microcirculation and the larger venous system. Most vessels within the microcirculation are lined by a layer of flattened endothelial cells. This endothelium provides a smooth surface for blood flow and regulates the movement of water and dissolved substances between the blood and the surrounding tissues, playing a central role in maintaining tissue homeostasis. Microvessels are integral to numerous physiological processes, including thermoregulation, immune response, and angiogenesis—the formation of new blood vessels. Dysfunctions in microvascular function are implicated in various pathological conditions, such as diabetes, hypertension, and cancer, underscoring their critical role in both health and disease.

The microcirculatory system is a complex network of branching vessels that spans a wide range of geometric scales and flow velocities [28]. Blood vessels in microcirculation are not simply straight tubes, in contrast, they are continually bifurcated and merged with other vessels resulting in complex networks [29, 30]. The vascular system comprises diverse geometries that range from normal (e.g. curves and bifurcations) to pathologic (e.g. aneurysms and stenoses), and the variations in the vascular geometry cause significant changes in the local shear stress profile during blood flow. Stroke, a major cause of mortality in SCD, is found to occur in cerebral arteries, which are highly tortuous [31]. The mechanics of blood flow in the circulatory system encompasses a diverse spectrum of fluid mechanical phenomena. In large blood vessels, with diameters ranging from millimeters to centimeters, inertial effects play a significant role. Phenomena such as boundary layers, flow separation, instability, secondary flows, and even turbulence may occur. However, in these microvessels, blood

behaves as a homogeneous Newtonian fluid, which serves as a simplifying assumption for fluid dynamic analyses. In the microcirculation, the situation is markedly different. Typical values [32, 33] of the wall shear rate $\dot{\gamma}_w$ in various vessels are $\sim 100 - 500 \text{ s}^{-1}$ in larger veins and arteries such as carotid, femoral, and coronary, 10^4 s^{-1} in arterioles, 10^3 s^{-1} in capillaries, and slightly lower in venules. In arteries with severe stenosis, shear rates as high as 10^5 s^{-1} can occur. The Reynolds numbers of blood flow are typically much less than 1, rendering inertial effects negligible. Under these conditions, the flow can be accurately described by the Stokes equations for an incompressible fluid, whose linearity provides a valuable simplification. Despite these simplifications, blood in microvessels cannot be treated as a homogeneous fluid due to its high concentration of suspended cells, primarily highly deformable RBCs. The dimensions of these cells are comparable to the diameters of the vessels, making noncontinuum effects highly significant. Thus it is crucial to understand the dynamics of a concentrated suspension of deformable particles within a geometrically complex network of branching microvessels.

The endothelium is a thin layer of specialized cells that line the interior surface of blood vessels, lymphatic vessels, and the heart. It serves as a critical interface between circulating blood or lymph and the surrounding tissues, playing a fundamental role in vascular biology [34]. Endothelial cells regulate a variety of essential functions, including controlling vascular tone through the release of vasodilators like nitric oxide and vasoconstrictors such as endothelin [35, 36]. They also mediate permeability, allowing selective exchange of nutrients, gases, and waste products [37]. Additionally, the endothelium plays a key role in maintaining hemostasis by balancing pro-coagulant and anti-coagulant factors, and it participates in immune responses by expressing adhesion molecules that recruit leukocytes to sites of inflammation [38–41]. Dysfunction of the endothelium is a hallmark of numerous pathological conditions, including atherosclerosis, hypertension, diabetes, and thrombosis, underscoring its importance in both health and disease [42–44].

The walls of living microvessels are lined with a layer of macromolecules attached to the

endothelial cells that form the inner surface of the vessel walls. This layer, known as the glycocalyx or endothelial surface layer, has been extensively studied [28, 45–47]. The thickness of the glycocalyx is variable but is estimated to be on the order of $1\text{ }\mu\text{m}$. From a mechanical perspective, this dilute, gel-like layer can be modeled as a deformable porous medium. The hydraulic resistance of the glycocalyx has been estimated to be at least 10^{11} N s/m^4 [48]. While this resistance is relatively low compared to other biological materials, it is sufficient to largely inhibit plasma flow within the layer. Although static RBCs can compress the glycocalyx, flowing RBCs are excluded by a lubrication-type mechanism, which has been compared to skiing on powder snow [49–51]. Endothelial cells are highly sensitive to wall shear stress, and the presence of the glycocalyx has significant mechanical implications. Specifically, the shear stress acting on the endothelial cell membrane is transmitted primarily by the glycocalyx rather than by the fluid directly adjacent to the membrane [50, 51]. In the context of blood flow in microvessels, the glycocalyx significantly reduces the effective diameter available for flow, thereby increasing flow resistance. This effect is critical for understanding microvascular hemodynamics. Despite its biological importance, the mechanics of interactions between suspended particles and a deformable porous wall layer, such as the glycocalyx, has received relatively little recent attention [52, 53].

This endothelial glycocalyx layer (EGL) is comprised of sulfated proteoglycans, hyaluronan (HA), glycoproteins, and plasma proteins that adhere to the surface matrix [47]. The EGL plays critical roles in regulating vascular function [47], including modulating permeability in transcapillary water exchange, acting as a mechanotransducer of fluid shear stress (FSS) to the endothelial cytoskeleton, initiating associated biochemical responses, and mediating interactions with RBCs and WBCs, particularly during inflammatory responses. The surface of endothelial cells (ECs) is decorated with a diverse array of membrane-bound macromolecules that form the EGL. These include glycoproteins bearing acidic oligosaccharides and terminal sialic acids (SAs), as well as proteoglycans with their associated glycosaminoglycan (GAG) side chains. The predominant GAGs associated with the vasculature are

heparan sulfate (HS), chondroitin/dermatan sulfate (CS), and hyaluronic acid (HA)[54, 55]. Among these, HS is the most abundant, comprising 50%–90% of the total GAG pool on EC surfaces, with the remainder consisting of CS and HA. HS and its associated proteoglycans play essential roles as signal transduction molecules [56–58]. HA, a much longer disaccharide polymer, contributes to the EGL’s hydration properties through its negatively charged carboxyl groups [59]. These groups enable HA to retain hydration properties and maintain structural integrity under physiological conditions. Complementing the GAG components, glycoproteins with short, branched oligosaccharides are also present on the EC surface. These oligosaccharides are capped by SAs—nine-carbon monosaccharides that contribute to the net negative charge of the EGL due to their ionization at physiological pH [60, 61].

The thickness of the EGL has been studied using many techniques. Smith et al. [62] used intravital near-wall micro-particle image velocimetry to estimate EGL hydrodynamic thickness ($\sim 0.3 - 0.35 \mu\text{m}$) based on velocity profiles near the vessel wall before and after EGL degradation. Damiano et al. [63] refined this approach with fluid dynamics analysis, validated in glass tubes and *in vivo* [64]. Their microviscometry method accounted for spatial shear rate variations, yielding a mean EGL thickness of $0.5 \mu\text{m}$ in control venules ($20 - 40 \mu\text{m}$ diameter) and $0.2 \mu\text{m}$ after EGL degradation. The flexural rigidity (E/I) of proteoglycan core proteins is a key parameter for understanding how FSS is transmitted from the EGL surface to the actin cortical cytoskeleton (ACC). Weinbaum et al. [65] estimated E/I indirectly, predicting the characteristic restoration time of the EGL after deformation by a WBC. Weinbaum’s model treats core proteins as elastic fibers anchored to the ACC by linker molecules. Hydrodynamic interactions between fibers are included, but entrained fluid motion is neglected. This model estimates E/I as 700 pN nm^2 . Han et al. [66] developed a more sophisticated large deformation elastic model, capturing time-dependent shape changes of core proteins from initial deformation to restoration after WBC passage. This model predicts E/I as 490 pN nm^2 , closely matching Weinbaum’s simpler linear elastic model [65].

The EGL plays a critical role in transmitting FSS to the actin cytoskeleton and initiating

intracellular signaling. Weinbaum et al. [65] proposed an elastohydrodynamic model where the EGL's core proteins have a flexural rigidity, EI , sufficient to resist deformation under physiological FSS. This rigidity allows the EGL to function as both a molecular sieve and a transducer of FSS, with predicted EI values of 500–700 pN nm² ensuring maximum fiber tip displacement of < 10 nm at 10 dyn/cm² FSS. Alternative models, such as the oncotic model [48, 51] and the mechano-electrochemical model [67], suggest that FSS is transmitted through axial tension in the GAG layer, which connects to transmembrane core proteins anchored to the endothelial cell cytoskeleton. Hydrodynamic drag on the GAGs induces tension, transmitting FSS into the cytoskeleton. Both models agree that FSS is converted into mechanical stress in the EGL and cytoskeletal elements, contrasting with the conventional view of direct skin-friction stress on an EGL-free endothelial cell lipid bilayer. The severe attenuation of flow within the EGL eliminates FSS on the endothelial cell surface, emphasizing the critical role of the EGL in FSS transmission.

The presence of the EGL in postcapillary venules [62, 64, 67] significantly impacts leukocyte recruitment and the inflammatory response cascade. Rolling leukocytes attach to vessel walls via selectin molecules that extend only 20–50 nm above the membrane [68], making de novo attachment (primary capture) unlikely, as even long leukocyte microvilli cannot penetrate the EGL unless the leukocyte is already tethered [69]. Experimental evidence confirms that primary capture is rare in inflamed microvessels [70], with secondary capture through leukocyte-leukocyte interactions being more common [71]. During tethered rolling, the penetration force, amplified by hydrodynamic drag, can reach 100 pN at a FSS of 1 dyn/cm² [69], enabling deep EGL penetration and sustained rolling. Most rolling leukocytes attach at postcapillary venule entrances [72, 73], where leukocytes exit deformed capillaries and compress the EGL, facilitating initial selectin-counterligand interactions [62, 65]. EGL compression by leukocytes can reach 80% in tightly fitting capillaries [66]. Inflammatory conditions may alter EGL properties [74], with cytokine-mediated protease activation degrading the EGL to localize leukocyte recruitment to inflamed tissue. This suggests the intact EGL acts as

a mechano-chemical barrier against recruitment in uninflamed regions. Localized degradation may represent a critical early step in inflammation, with therapeutic inhibition of EGL shedding offering potential for regulating inflammation. Studies of tethered rolling in parallel-plate flow chambers often lack intact EGLs, focusing instead on selectin-ligand kinetics [62].

1.3 Rheological Properties of Blood

Macroscopic viscosity measurements of blood demonstrate that it behaves as a shear-thinning fluid with a yield stress [75]. At 37°C, blood viscosity reaches a constant value of approximately 3 – 5 mPa s when the wall shear rate $\dot{\gamma}_w$ exceeds 100 s⁻¹, with a yield stress of about 4 mPa. At 20°C, the viscosity plateaus at high shear rates to a value of approximately 10 mPa s. These values depend on factors such as temperature and hematocrit. Plasma, the liquid component of blood, is generally considered Newtonian, with a viscosity ranging from 1.3 mPa s to 2 mPa s. Evidence suggests that plasma exhibits some viscoelastic behavior, characterized by a relaxation time of approximately 2 ms [76]. The shear-thinning behavior of blood is primarily attributed to the deformability of RBCs under flow. In contrast, suspensions of chemically stiffened RBCs do not exhibit shear thinning [77]. Additionally, blood exhibits viscoelastic properties, again largely due to the deformability of RBCs [78]. The yield stress of blood is relatively small, with any flow exhibiting a shear rate $\dot{\gamma}$ of approximately 1 – 10 s⁻¹ being sufficient to overcome it. It has long been observed that RBCs in stagnant blood form rouleaux—aggregates in which RBCs stack together in a coin-like arrangement. Although the precise mechanisms underlying rouleaux formation are not fully understood, depletion flocculation driven by the abundance of albumin and globulin molecules in plasma, appears to play a substantial role [79, 80].

At flow geometries with scales comparable to those of individual blood cells, Fåhræus and Lindqvist [81] made a seminal observation regarding the apparent viscosity of blood

at shear rates $\gtrsim 100 \text{ s}^{-1}$. This shear rate corresponds to the constant-viscosity plateau observed beyond the shear-thinning region. Using a capillary viscometer and Poiseuille's law, they demonstrated that the apparent viscosity of blood strongly depends on the diameter of the capillary tube [8]. This phenomenon, now termed the Fåhræus-Lindqvist effect, underscores the complex interplay between blood flow and vessel geometry. For large tubes, the apparent viscosity asymptotically approaches a plateau. However, within the range of tube diameters from $10 \mu\text{m}$ to $1000 \mu\text{m}$, the apparent viscosity decreases significantly as the tube diameter diminishes. For tubes smaller than $10 \mu\text{m}$, the viscosity increases sharply. The rise in viscosity at very small diameters is readily attributed to the fact that RBCs, with a typical diameter of approximately $8 \mu\text{m}$, experience considerable confinement in such narrow geometries. This confinement amplifies the influence of individual cell dynamics on flow behavior. In contrast, the viscosity reduction at intermediate tube diameters is more intricate, requiring a detailed understanding of the interactions between RBCs and the vessel wall.

The origin of this phenomenon is closely related to another classical observation in blood physiology, known as the Fåhræus effect [82]. This effect describes the observation that the hematocrit of blood within a tube—referred to as the "tube hematocrit", representing the true average volume fraction of RBCs—can be lower than the "discharge hematocrit", which is inferred from measurements of the blood exiting the tube. The ratio of tube hematocrit to discharge hematocrit exhibits a dependence on tube diameter that is similar to the behavior of apparent viscosity. Notably, in tubes with a diameter of approximately $20 \mu\text{m}$, the tube hematocrit is observed to be about half of the discharge hematocrit [33, 82]. The resolution to this apparent paradox lies in the influence of the nearly parabolic velocity profile within the tube. The discharge hematocrit is weighted by this velocity profile, while the tube hematocrit is not. If the local concentration of RBCs within the tube were uniform, these two measures would yield identical results. However, the Fåhræus effect reveals that the RBC concentration is nonuniform, with a higher concentration near the center of the channel,

where velocity is greatest, and a lower concentration near the walls, where velocity is minimal. This variation in concentration arises from two primary mechanisms. The first is a simple volume exclusion effect: RBCs cannot pass through the vessel wall, resulting in a region near the wall with a cell volume fraction lower than the nominal value. The second, more intriguing mechanism, involves the migration of RBCs and other deformable particles away from the walls during shear flow, driven by hydrodynamic interactions with the walls. This migration creates a cell-depleted region near the vessel walls, referred to as the "cell-free layer" [83, 84].

An important consequence of the nonuniform concentration of RBCs during flow is the phenomenon known as "plasma-skimming". In the highly branched structure of the circulatory system, the cell-depleted region near the vessel walls results in a lower hematocrit in daughter vessels compared to the parent vessel. This occurs because daughter vessels disproportionately draw fluid from the wall regions of the parent vessel, which are relatively depleted of cells [83, 85]. Closely related to plasma-skimming is the Zweifach-Fung effect, which describes the unequal distribution of RBCs at asymmetric bifurcations. Specifically, when a suspension of particles encounters such a bifurcation, the RBC volume fraction is higher in the outlet branch with the greater flow rate compared to the branch with the lower flow rate [86, 87]. This effect primarily arises from the cell distribution in the inlet branch. Similar to plasma-skimming, the presence of the cell-free layer strongly influences the RBC distribution in the outlet branches [88]. *in vivo* measurements in small blood vessels provide further evidence of the substantial reduction in tube hematocrit that results from the combined effects of Fåhræus and plasma-skimming phenomena [33].

1.4 Cells Segregation and Margination During Blood Flow

The cross-stream distribution of blood cells in microcirculatory flow exhibits complex and nontrivial behavior. Even at zero Reynolds number, deformable objects in shear flow—such as capsules, drops, vesicles, or flexible macromolecules—migrate away from the vessel walls [89–92]. In particular, RBCs tend to migrate away from the walls due to the fluid motion generated as they attempt to relax to their rest shape [90, 93]. This migration results in the formation of a cell-depleted region near the vessel walls, commonly referred to as the “cell-free layer” [83, 84]. Additionally, in Poiseuille flow, where the velocity gradient is nonuniform, a single deformable particle can undergo lateral migration even in the absence of hydrodynamic wall effects [90]. If the Reynolds number is finite, there is also an inertial lift force [94, 95], which can be important in microfluidic applications at a relative high flow rate. In suspensions, this migration is counterbalanced by shear-induced diffusion, a phenomenon driven by the random motion of particles caused by fluid disturbances generated by neighboring particles. Notably, collisions between neighboring particles contribute significantly to this effect. The shear-induced diffusivity of particles in a dilute suspension is expected to be proportional to the product of the collision frequency and the mean squared displacement per collision.

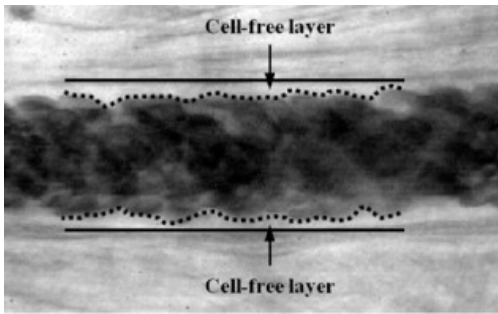
WBCs and PLTs exhibit behavior distinct from that of RBCs, preferentially migrating toward the vessel walls in a phenomenon known as margination [29, 96]. This nonuniform distribution is of physiological significance, as the primary functions of WBCs and PLTs, such as mediating inflammatory responses and promoting hemostasis, are closely linked to their interactions with the vessel wall. Experimental studies, conducted both *in vivo* and *in vitro*, have characterized the margination behavior of leukocytes and observed a strong dependence on shear rate [96–99]. At shear rates $\gtrsim 100 \text{ s}^{-1}$, the margination of leukocytes tends to decrease with increasing shear rate. This trend may be partially explained by the reduction

in RBC aggregation at higher shear rates. For example, inducing RBC aggregation through the addition of dextran, which promotes depletion flocculation, has been shown to enhance margination [96, 98]. The decrease in aggregation as shear rate increases may thus contribute to the observed reduction in margination [99]. The effect of hematocrit on margination is less straightforward. While some studies report that margination is either unaffected by hematocrit or increases slightly within experimental errors [99], more recent investigations have revealed a nonmonotonic relationship. Specifically, a maximum in margination has been observed at a hematocrit of $\phi = 0.2$ [100]. This nonmonotonic dependence has also been corroborated by computational models, further highlighting the complex interplay between hematocrit and margination behavior. The effect of channel width on leukocyte margination has also been examined, with findings generally indicating that the degree of margination decreases as channel width increases. *in vitro* studies investigating sudden expansions in channel geometry suggest that optimal margination occurs within channel widths in the range of $25 - 50 \mu\text{m}$ [101].

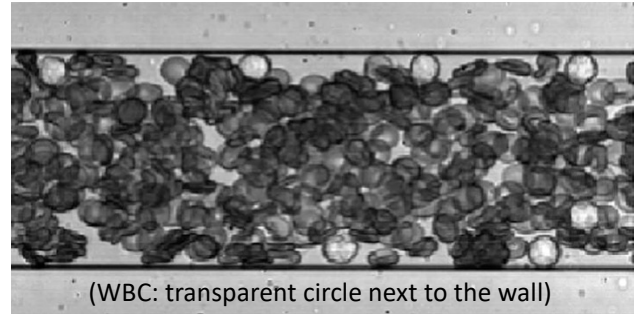
The margination behavior of platelets in blood flow has been studied through both *in vivo* and *in vitro* experimental investigations [102–105]. These studies generally report that platelet margination increases with both hematocrit and shear rate. However, some findings suggest a more complex, nonmonotonic dependence on shear rate [105]. Computational studies have further explored the mechanisms underlying platelet margination. One proposed mechanism involves the platelet drift toward the wall at the edge of the red-cell-free layer, driven by asymmetric collisions with RBCs [106]. Alternatively, other researchers have argued that higher velocity fluctuations near the center of the vessel, where hematocrit is greater, propel platelets toward the vessel walls, where these fluctuations are minimal. This redistribution contributes to their margination [106]. Another important factor influencing platelet margination is the size disparity between platelets and RBCs. Simulations have shown that replacing RBCs with platelet-sized, yet still deformable, particles eliminates platelet margination [107]. This finding highlights the critical role of size differences and

mechanical interactions in determining platelet distribution within the flow field.

The segregation behavior during blood flow, the discrepancy in the spatial distribution of different species of blood cells, is substantially dictated by the aberrance in cellular properties, such as shape, size, and deformability. Numerous computational efforts have been made in the past decade using direct simulations to understand the individual roles of various physical properties on the segregation behavior in confined flowing multicomponent suspensions. Kumar *et al.* [108, 109] probed the effect of rigidity difference in a binary suspension of deformable capsules subjected to either simple shear flow or pressure-driven flow in a planar slit. They found that stiff capsules display substantial margination when they are dilute, while flexible capsules tend to enrich around the centerline of the channel. The effect of size contrast was also revealed [109]: in a mixture of large and small capsules with equal capillary number, the smaller capsules marginate while the large particles demarginate. Likewise, Sinha *et al.* [110] investigated the flow-induced segregation behavior in binary suspensions of spherical and ellipsoidal capsules in simple shear flow by varying the aspect ratio while keeping constant either equatorial radius or volume of capsules.



(a) Formation of the cell free layers [111].



(b) Margination of white blood cells [112].

Figure 1.1: In the microcirculation, all types of blood cells display a nonuniform distribution across the vessels. Normal RBCs migrate away from the vessel walls leading to the formation of cell free layers, while white blood cells marginate towards the vascular walls.

Furthermore, a number of phenomenological theories have been developed to elucidate the segregation behavior from a mechanistic perspective [109, 113–116]. These theories have been rooted in taking into account two key sources of cross-stream particle fluxes in flow-

ing suspensions under confinement: wall-induced migration flux away from walls, which is ubiquitous in multicomponent suspensions, and shear-induced diffusive flux due to hydrodynamic pair collisions between surrounding particles. For instance, a mechanistic model, given by Henríquez Rivera *et al* [115], consisting of a set of simplified drift-diffusion (SDD) equations, has been developed to describe the dynamics of confined flowing multicomponent suspensions. The predictions of cell-free layer thickness in their work were consistent well with the results from other experiments, detailed simulations, and theories. Moreover, the rising of several regimes of segregation, in either simple shear flow or pressure-driven flow, is found to depend on the value of a so-called "margination parameter" M , a determinant of the segregation propensity that characterizes the relative significance of the wall-induced migration and the collision fluxes. Moreover, a sharp “drainage transition”, indicated by the complete depletion of one component from the bulk flow to the vicinity of the walls, is predicted when M is below some critical value.

1.5 Biomechanics and Endothelial Damage in Blood Disorders

Disorders affecting RBCs encompass a wide spectrum of conditions with significant implications for human health, underscoring the importance of understanding their underlying mechanisms and consequences. A notable example is sickle cell disease (SCD), a monogenic hemoglobin disorder characterized by stiffened, sickle-shaped, or otherwise deformed RBCs. This condition leads to chronic pain, organ damage, and potentially life-threatening complications [117]. The recent COVID-19 pandemic, caused by SARS-CoV-2 infection, has also been associated with substantial alterations in RBCs. Emerging evidence suggests that severe COVID-19 may result in endothelial damage and microvascular thrombosis, contributing to organ dysfunction [118]. Similarly, sepsis, a life-threatening condition triggered by severe systemic infection, disrupts RBC morphology and function, leading to organ failure, hy-

potension, and elevated mortality rates [119]. Iron deficiency anemia, another prevalent RBC disorder, impairs RBC production and oxygen-carrying capacity, exerting deleterious effects on the cardiovascular system [120]. Hereditary spherocytosis, characterized by abnormally spherical-shaped RBCs, compromises cell membrane stability and increases susceptibility to hemolysis [121]. A comprehensive investigation into these RBC disorders is essential for advancing our understanding of their complexities and the critical importance of maintaining RBC health and functionality. Such insights are vital for mitigating vasculopathy and improving clinical outcomes for affected individuals.

Aberrant RBCs arising from blood disorders often exhibit markedly different physical properties compared to healthy RBCs. A typical example is SCD, in which cell-level alterations in the physical properties of diseased RBCs lead to aberrant hemodynamics and further induce complications. Under deoxygenated conditions, HbS molecules polymerize into long rigid fibrils within RBCs, a process termed sickling. These intracellular fibrils mechanically alter and damage the membrane of RBCs, resulting in the classic “sickled” or crescent-like shape for sickle cells [122]. In fact, in addition to this characteristic sickle shape, a large variation in cell morphology has long been observed within the population of sample sickle cells. Kual *et al.* [123] revealed the effect of deoxygenation rate on the morphological characteristics of sickle cells. Byun *et al.* [124] performed optical measurements for individual sickle RBCs and classified them into echinocytes, discocytes, and the typical crescent-shaped irreversibly sickled cells (ISC) based on their morphological features. Here, ISCs commonly refer to those subjected to irreversible membrane damage after repeated cycles of intracellular sickling that have lost membrane elasticity and remain sickle-shaped even when exposed to extensive oxygenation [125, 126]. In SCD, abnormal sickle hemoglobin polymerizes within RBCs upon deoxygenation, forming long fibers that pathologically disrupt cellular architecture [127]. The intracellular sickling also results in substantial alterations in the mechanical properties of the cell membrane. Using various techniques for the characterization of cell membrane mechanics, a general increase in membrane stiffness of sickle cells compared to

that of healthy RBCs has been experimentally determined [124, 128]. Specifically, the shear modulus for ISCs is determined to be three to four times that for healthy RBCs, though no substantial difference in shear modulus is found between healthy RBCs and non-ISCs [124]. Besides, SCD patients have a small population (1-10%) of sickle RBCs due to the much shorter life span (~ 15 days) than healthy RBC (~ 115 days) [129]. In addition, ISCs also have a much smaller volume than healthy RBCs due to cellular dehydration [130]. This disruption leads to increased membrane stiffness and cellular dehydration, resulting in a loss of cell volume. Consequently, sickle RBCs are biophysically less deformable than healthy cells, with some subpopulations irreversibly distorted into a sickle-like shape. In iron deficiency anemia (IDA), Caruso et al. [131] identified a subpopulation of RBCs, termed iron-deficient RBCs (idRBCs), that are significantly smaller and poorly deformable. Similarly, during COVID-19 infection, morphologically normal RBCs undergo a conformational change into spherocytosis, characterized by reduced size and impaired deformability [132]. Furthermore, plasma from adult COVID-19 patients has been shown to induce substantial RBC aggregation under flow, driven by fibrinogen-mediated interactions. This aggregation causes direct damage to the endothelial glycocalyx, exacerbating vascular dysfunction [118]. In hereditary spherocytosis (HS), genetic mutations disrupt the linkage between the membrane skeleton and lipid bilayer of RBCs, resulting in membrane loss [121]. These alterations transform the characteristic biconcave discoid shape of RBCs into inflexible spherical cells, termed spherocytes. These structural and biophysical changes in RBCs significantly compromise their deformability, functionality, and interactions within the vasculature, contributing to the pathophysiology of these blood disorders and their associated clinical manifestations.

The substantial advancements in understanding the underlying mechanisms of flow-induced segregation in multicomponent suspensions have provided a foundation for investigating suspension dynamics in more complex scenarios. We hypothesize that purely mechanical interactions between sickle RBCs and the endothelium, resulting from the margination of stiff diseased RBCs, play a significant role in driving endothelial inflammation in SCD.

Although adhesive RBC-endothelial interactions, inflammatory cytokines, and hemolysis all contribute to SCD vasculopathy, Tsai *et al.* [133] developed an *in vitro* microvasculature model to specifically investigate the role of cellular interactions in hematological diseases. This model consisted of endothelial cells cultured on the inner surface of a microfluidic system. Their study revealed that Vascular Cell Adhesion Molecule 1 (VCAM-1), a biomarker of endothelial dysfunction, was upregulated when exposed to flowing stiffened RBCs compared to normal RBCs [134]. These findings confirmed that stiffer RBCs marginate toward blood vessel walls under physiologic flow conditions. Furthermore, Caruso *et al.* [1] extended this work by perfusing suspensions of SCD RBCs and nystatin-stiffened RBCs into endothelialized microfluidic channels. Normal RBCs were treated with nystatin to increase cellular stiffness, effectively decoupling hemolytic and adhesive effects from purely mechanical interactions. Their results showed that endothelial inflammation increased monotonically with the fraction of sickle RBCs in the suspension. However, for nystatin-stiffened RBCs, the relationship was nonmonotonic, with maximum endothelial damage observed at an intermediate fraction of stiffened cells (Fig. 1.2(C)). These findings suggest that purely mechanical interactions between endothelial cells and SCD RBCs are sufficient to induce inflammation. Notably, the observation that heterogeneous RBC populations with a small minority of stiff cells cause greater inflammation than uniformly stiff RBCs warrants further investigation.

The vascular system encompasses a wide range of geometries, spanning from normal structures (e.g., curves and bifurcations) to pathological anomalies (e.g., aneurysms and stenoses). Variations in vascular geometry significantly alter the local shear stress profile during blood flow, which is known to induce endothelial proinflammatory responses [135]. Using an endothelialized microfluidic model with multiple geometric configurations, Manino *et al.* [2] demonstrated that VCAM-1 expression correlated strongly with shear stress variation and was most pronounced near bifurcation points (Fig. 1.2(D)). Additionally, Wang *et al.* [3] observed that endothelial cells exposed to SCD RBCs (sickle cell disease red blood cells) and nystatin-stiffened RBCs exhibited increased inflammation along the outer wall of

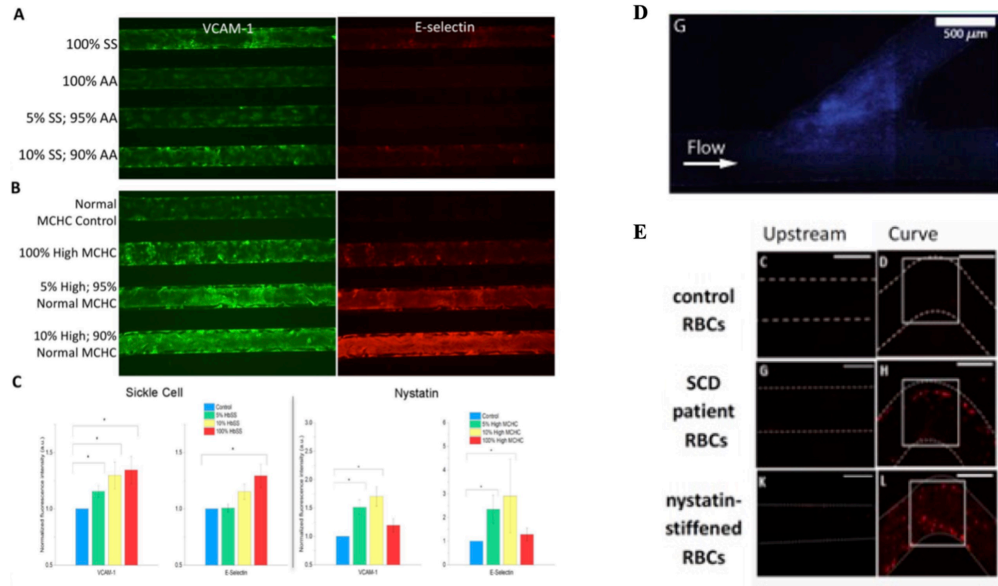


Figure 1.2: Experimental observations of SCD induced endothelial damage in the endothelialized microfluidics [1–3]. VCAM-1(green) and E-selectin(red) expression in microfluidics of (A) normal vs SCD RBCs and (B) normal vs nystatin-stiffened RBCs. (C) Mean normalized fluorescent intensity for VCAM-1 and E-selectin expression in (left) normal vs SCD RBCs and (right) normal vs nystatin-stiffened RBCs. (D) VCAM1 expression within a vascular bifurcation. (E) SCD and nystatin-treated RBCs upregulate E-selectin at the curvature site of microfluidics.

curved regions in vessels. This phenomenon is attributed to regions of high wall shear stress along the outside wall of curved vessels, where stiff SCD RBCs preferentially marginate due to inertial effects generated by fluid flow around a bend. Moreover, endothelial cells exposed to artificially stiffened RBCs displayed significantly elevated diffuse VCAM-1 and E-selectin expression throughout the entire curved region compared to healthy and SCD RBCs (Fig. 1.2(E)). This enhanced expression likely arises from the higher degree of RBC margination associated with artificially stiffened cells compared to SCD RBCs. Collectively, these findings confirm that both vascular geometry and the mechanical interactions between stiffened RBCs and the endothelium play critical roles in the development of SCD vasculopathy.

In recent years, our group has aimed to take an initial step toward computationally understanding the dynamics of blood suspension in SCD. From a purely physical perspective, pathological alterations in the properties of diseased cells were incorporated into numerical

models to uncover the mechanisms underlying chronic endothelial inflammation in SCD. At the single-cell level, Zhang *et al.* [136, 137] investigated the orbital dynamics of isolated deformable curved and straight prolate fluid-filled capsules in unbounded simple shear flow under the Stokes regime. The curved prolate capsule served as a model for the characteristic crescent-shaped sickle cells in SCD. Their study revealed that, over long timescales, the dynamics of the capsule always evolved into a Jeffery-like quasiperiodic orbit regardless of its initial orientation. The average behavior of this long-term trajectory could be accurately predicted using the analytical solution for Jeffery orbits with an effective orbit constant C_{eff} and aspect ratio l_{eff} . Additionally, for capsules with low deformability, straight prolate spheroidal capsules exhibited tumbling in the shear plane as their unique asymptotically stable orbit. These findings illustrate how the aberrant mechanical properties and distorted shape of sickle cells lead to distinct trajectories, potentially contributing to pathological activation of other cells, and lay the groundwork for studying more complex systems. Building upon these insights, Zhang *et al.* [138] performed detailed boundary integral simulations to investigate the dynamics of a binary suspension of flexible biconcave discoid fluid-filled capsules and stiff curved prolate capsules, representing healthy and sickle RBCs, respectively. This system modeled microvascular blood flow in SCD, confined within a planar slit under pressure-driven flow. A key observation was that, unlike healthy RBCs, which concentrated near the channel center and formed an RBC-depleted layer near the walls, sickle cells were largely drained from the bulk suspension and enriched within the cell-free layer, exhibiting pronounced margination behavior. Furthermore, binary suspensions displayed significantly larger local peaks in wall shear stress compared to the relatively small fluctuations observed with purely healthy RBCs. These peaks were attributed to the proximity of marginated stiff cells to the walls. These results support the hypothesis that purely physical, non-adhesive interactions between sickle cells and the endothelium are sufficient to cause chronic endothelial dysfunction.

The aberrations in cellular properties in the context of SCD pathologically alter blood

flow dynamics in the microcirculation. We hypothesize that these altered hemodynamics promote the margination of stiff sickle cells, thereby enhancing mechanical interactions between blood cells and vascular walls, which are sufficient to drive vasculopathy in SCD. To investigate the mechanisms underlying endothelial inflammation associated with SCD, Zhang *et al.* [138] performed direct simulations of a binary suspension of flexible biconcave discoid capsules and stiff curved prolate capsules, representing normal and sickle RBCs, respectively, under pressure-driven flow in a planar slit. This model serves as an idealized representation of microvascular blood flow in SCD. However, a notable limitation of this model is the assumption that all sickle cells share identical rest shapes and membrane mechanics. In reality, clinical observations reveal significant heterogeneity in these physical properties among the population of sickle cells in SCD patients [124]. Elucidating the effects of cell and suspension properties—such as shape, size, stiffness, adhesiveness, and polydispersity—on spatial distributions and cell-wall interactions during blood flow is therefore a priority. The current study is motivated by experimental findings that suggest complex interactions in heterogeneous RBC populations. Numerical simulations will be employed to gain detailed insights into these phenomena. For example, Caruso *et al.* [1] demonstrated that heterogeneous RBC populations containing a small fraction of stiff cells induce greater endothelial inflammation than populations with uniformly stiff RBCs. Additionally, Passos *et al.* [139] examined the effects of impaired RBC deformability on velocity and hematocrit distributions in microscale blood flow. Their experiments, which involved perfusing suspensions of healthy and glutaraldehyde-hardened RBCs through straight microchannels under varying hematocrits and flow rates, revealed that dense suspensions of hardened RBCs exhibited sharper hematocrit profiles compared to healthy cells, suggesting a central concentration of hardened RBCs within the channel. These counterintuitive results were attributed to flow alignment, ordering effects, and screened wall-induced lift forces. Given the potential role of ISC adhesiveness in altered hemodynamics, the effect of cellular interactions will be incorporated into the simulations. Cell aggregation will be modeled using the Morse potential, as

proposed by Liu *et al.* [140, 141], which has been extensively applied to simulate RBC interactions. For ISC adhesion to the vascular wall, adhesion potential energy will be modeled by incorporating receptor-ligand binding kinetics using a stochastic framework [142–144]. RBCs in SCD patients exhibit significant heterogeneity in size, shape [124], and mechanical properties [124, 128], which vary with the oxygenation state of the blood [123]. A key objective of this work is to identify robust phenomena and parameter regimes that remain consistent despite these variabilities, with particular attention to the effects of polydispersity on observed phenomena.

The propensity of RBCs to aggregate is a fundamental phenomenon that significantly influences blood flow and circulation. RBC aggregation is primarily mediated by interactions between surface proteins on RBCs and various plasma proteins, particularly fibrinogen. Fibrinogen, a glycoprotein abundant in plasma, acts as a molecular bridge between adjacent RBCs, promoting adhesion and aggregation. Additional factors, including cell surface charges and the presence of specific plasma constituents, further modulate the degree of aggregation. While a baseline level of RBC aggregation is physiologically normal, aberrant aggregation has profound implications for hemodynamics and cardiovascular health. Notably, abnormal RBC aggregation has been associated with endothelial dysfunction and the induction of a pro-inflammatory state in endothelial cells lining the vasculature. Such conditions are characteristic of various disorders, including COVID-19, sepsis, and sickle cell disease, and have been linked to an elevated risk of stroke and other severe circulatory complications [118, 145–151]. Understanding the mechanisms underlying RBC aggregation and its interactions with the vascular endothelium is therefore critical for addressing the pathological consequences associated with these conditions.

Recent advances in experimental models have provided significant insights into the pathological impact of RBC aggregation. Druzak *et al.* [118] utilized a microfluidic microvasculature model to demonstrate, for the first time, that elevated fibrinogen levels—leading to an increased propensity for RBC aggregation—directly damage the endothelial glycocalyx. The

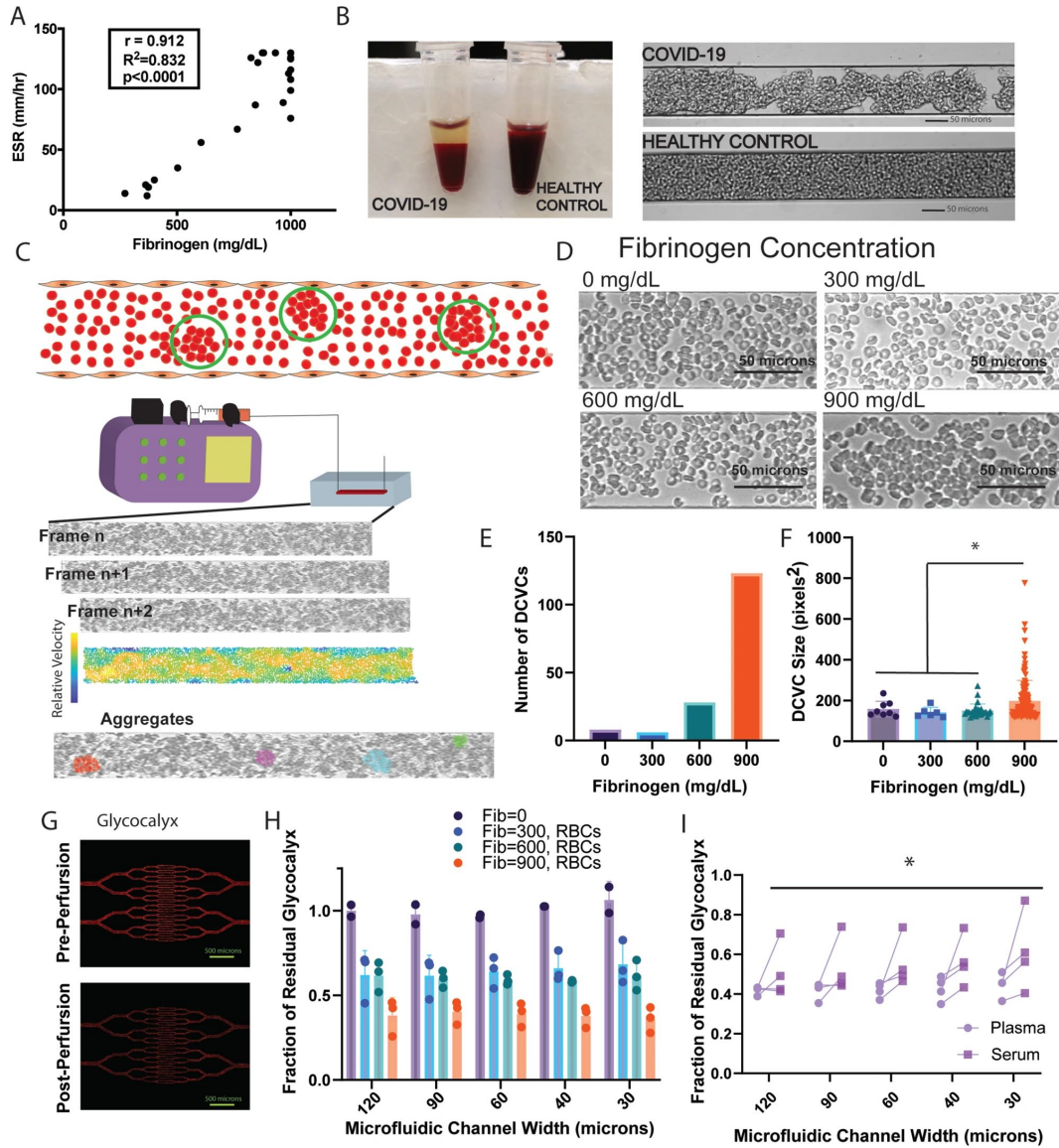


Figure 1.3: Fibrinogen mediates RBC aggregation and biophysically induces endothelial cell damage [4]. (A) Positive correlation between ESR and fibrinogen levels in COVID-19 patients. (B) (Left) COVID-19 patient blood shows rapid sedimentation on the benchtop and (Right) aggregation in a microfluidics channel under static conditions compared with healthy control. (C) RBC aggregation detected using our velocity field calculations and the differential cell velocity cluster (DCVC) identification. (D) Frames showing a qualitative increase in RBC aggregation with increasing fibrinogen. (E) Exponential increase in the number of detected DCVCs with increasing fibrinogen level. (F) Increase in the mean DCVC size with increasing fibrinogen concentration. (G) Endothelial glycocalyx in serially branching microvasculature-on-chip devices stained with fluorescently tagged wheat germ agglutinin before (top) and after (bottom) perfusion of RBCs. (H) Average fraction of residual glycocalyx detected in the microfluidics devices. (I) Measurement of glycocalyx degradation.

glycocalyx, a glycoprotein layer that coats the endothelial cell surface, plays an essential role in maintaining vascular integrity. Their experiments revealed that RBC aggregation became progressively more pronounced with increasing fibrinogen concentrations (Fig. 1.3(D)). To quantify RBC aggregation, computer vision techniques were employed to analyze the number and size of RBC clusters formed during blood flow (Fig. 1.3(C)). This analysis demonstrated a significant increase in both the number and size of RBC clusters at higher fibrinogen levels (Fig. 1.3(E,F)). Building on these findings, the effects of RBC aggregation on the vascular surface were examined. RBC suspensions with varying fibrinogen concentrations were perfused through endothelialized microfluidic channels (Fig. 1.3(G)). Measurements of the endothelial glycocalyx layer before and after perfusion revealed a marked reduction in the glycocalyx fraction in cases involving fibrinogen-induced RBC aggregation. Moreover, higher fibrinogen concentrations were correlated with more severe vascular damage (Fig. 1.3(H,I)). These results provide compelling evidence that fibrinogen-induced RBC aggregation exerts deleterious effects on both the endothelium and the glycocalyx layer, underscoring its detrimental impact on vascular health. In summary, these findings highlight the critical role of fibrinogen-induced RBC aggregation in vascular damage, emphasizing its contribution to blood flow-related pathologies. A deeper understanding of the mechanisms underlying RBC aggregation and its impact on vascular integrity is vital for advancing therapeutic strategies aimed at mitigating its adverse effects and improving outcomes in related disorders.

2

Marginated aberrant red blood cells
induce pathologic vascular stress
fluctuations in a computational model
of hematologic disorders ¹

¹The text of this chapter is adapted from the publication by X. Cheng, C. Caruso, W. A. Lam, and M. D. Graham. *Science Advances*, 9.48, 2023

Red blood cell (RBC) disorders such as sickle cell disease affect billions worldwide. While much attention focuses on altered properties of aberrant RBCs and corresponding hemodynamic changes, RBC disorders are also associated with vascular dysfunction, whose origin remains unclear, and which leads to severe consequences such as stroke. Little research has explored whether biophysical alterations of RBCs affect vascular function. Here we employ a detailed computational model of blood that enables characterization of cell distributions and vascular stresses in several blood disorders. Our simulation results are compared with experimental observations. Aberrant RBCs, with their smaller size and higher stiffness, concentrate near the vessel walls (i.e. marginate) due to contrasts in physical properties relative to normal cells. In a curved channel that exemplifies the geometric complexity of the microcirculation, the distribution of marginated cells is very heterogeneous, indicating the importance of geometry. The marginated cells generate large transient stress fluctuations on the vessel walls, indicating a mechanism for the observed vascular inflammation.

2.1 Introduction

Disorders that affect red blood cells (RBCs) encompass a diverse range of conditions with substantial implications for human health, highlighting the need to comprehend their underlying mechanisms and effects. One prominent example is sickle cell disease, a monogenic hemoglobin disorder resulting in stiffened and sickle-shaped or otherwise deformed RBCs. Sickle cell disease leads to chronic pain, organ damage, and life-threatening complications [117]. In addition, the recent global pandemic of COVID-19, caused by SARS-CoV-2 infection can lead to substantial alterations of RBCs [132]. Emerging evidence suggests that organ dysfunction associated with severe COVID-19 may result from endothelial damage and microvascular thrombosis [118]. Sepsis, a life-threatening condition arising from severe systemic infection, disrupts the morphology and function of RBCs, precipitating organ failure, hypotension, and increased mortality rates [119]. Iron deficiency anemia, adversely affects

RBC production and their oxygen-carrying capacity, consequently exerting detrimental effects on the cardiovascular system [120]. Hereditary spherocytosis, which is characterized by abnormal spherical-shaped RBC, affects cell membrane stability and increases susceptibility to hemolysis [121]. A comprehensive exploration of these RBC disorders will enable a deeper understanding of their intricacies and the crucial significance of maintaining RBC health and functionality and alleviating vasculopathy.

In individuals with blood disorders, endothelial cells lining the blood vessels are often dysfunctional and in a pro-inflammatory state, increasing the risk of stroke and atherosclerosis [147–151]. In particular, stroke, a predominant cause of mortality in sickle cell disease (SCD), often occurs in highly tortuous cerebral arteries and is associated with endothelial inflammation and chronic vasculopathy. In patients with cardiovascular disease and iron deficiency anemia (IDA), improved disease outcomes were observed with iron supplementation and subsequent resolution of IDA.[152]; however, the underlying pathophysiologic basis for the association remains unknown. The interplay among adhesive RBC-endothelial interactions, inflammatory cytokines, and hemolysis all contribute to vasculopathy in blood disorders, however the potential contribution of the altered physical properties of aberrant RBCs, particularly shape and stiffness, to the hemodynamic environment experienced by the vascular endothelium remains poorly understood. This topic is the focus of the present work.

Vascular geometries contribute to vasculopathy in blood disorders [153]. The vascular system is comprised of diverse geometries, including normal complexities such as curves and bifurcations as well as pathologic ones such as aneurysms and stenoses, and variations in vascular geometry cause substantial changes in the local shear stress profile during blood flow, which are known to induce endothelial proinflammatory responses [135, 148, 150, 153]. Leveraging an endothelialized microfluidic model of multiple geometries, Mannino et al. [2] found that VCAM-1 and E-selectin expression, biomarkers of endothelial cell dysfunction, significantly correlated with shear stress variation and were most pronounced near bifurcation

points. Furthermore, they found that endothelial cells exposed to SCD RBCs exhibited increased endothelial inflammation along the outside wall of the bend in the curved regions of vessels [3]. These observations indicate that it is essential to understand the role of vascular geometric complexity on endothelial dysfunction in blood disorders.

Aberrant RBCs arising in blood disorders often have very different physical properties compared to healthy RBCs. A typical example is SCD, in which abnormal sickle hemoglobin polymerizes within RBCs upon deoxygenation, creating long fibers that pathologically disrupt cellular architecture[127], leading to increased membrane stiffness as well as loss of cellular volume secondary via dehydration. Subsequently, sickle RBCs are biophysically less deformable than normal cells and some subpopulations are distorted irreversibly into a sickle-like shape. Similarly, in samples of blood from IDA patients, Caruso et al.[131] identified a subpopulation of very small and poorly deformable iron deficiency RBCs (idRBCs). When exposed to COVID-19, morphologically normal RBCs exhibited a conformational change to spherocytosis with reduced size and deformability [132]. Relatedly, plasma from adult COVID-19 patients causes substantial RBC aggregation under flow, and fibrinogen-mediated aggregation directly damages the endothelial glycocalyx [118]. In hereditary spherocytosis (HS), genetic mutations affect RBC membrane proteins, breaking the linkage between the membrane skeleton and the lipid bilayer, causing membrane loss [121]. As a result, instead of being biconcave discs, RBCs become inflexible spherical cells called spherocytes.

The spatial distribution of the different cellular components of blood is nontrivial and depends on the relative physical properties of the different components. Normal RBCs migrate toward the center of a blood vessel, leaving an RBC-depleted cell-free layer (CFL) near vessel walls. In contrast, white blood cells (WBCs) and platelets tend to reside in these layers, a flow-induced segregation phenomenon called margination [96, 102, 154]. Experimental observations of modifications to cell segregation in disease are rare. Observing the flow of suspensions comprised of SCD RBC populations of two different densities, Clavería et al.[155] investigated whether segregation occurs among SCD RBCs flowing in micron-sized channels.

It is known [156] that SCD RBCs with higher density exhibited greater shear modulus and, consequently increased rigidity. Clavería et al. found a heterogeneous distribution of SCD RBC according to their density: low-density SCD RBC population remained closer to the center of the channel, while the densest (i.e. stiffest) cells were segregated towards the walls.

The segregation behavior during blood flow is substantially dictated by the contrasts in the cellular properties, such as shape, size, and deformability, of the various components. Kumar et al. [108, 109] used detailed simulations to probe the effect of rigidity difference in a binary suspension of deformable capsules in shear flow. They found that stiff capsules display substantial margination when they are the dilute component, while flexible capsules tend to enrich around the channel’s centerline. Similarly, in a mixture of large and small capsules, the smaller capsules marginate [109]. Sinha et al. [110] investigated the flow-induced segregation behavior in binary suspensions of spherical and ellipsoidal capsules in simple shear flow by varying the aspect ratio while keeping constant either the equatorial radius or volume of capsules. Direct simulations with models of blood corroborate these model results [157, 158]. A simple theory of margination based on the two key transport mechanisms of cells in flow – cell-cell collisions and hydrodynamic migration of deformable cells away from walls [159, 160] predicts that a subpopulation of rigid particles in a suspension of primarily deformable particles, will strongly concentrate at walls during flow [114, 115].

Margination may have particular significance in the context of vasculopathy in blood cell disorders. Endothelial cells are responsible for translating biophysical cues, such as the shear force of the hemodynamic microenvironment, into cellular biological signals [151, 161, 162]. Pathological alterations of such forces promote endothelial activation with the release of proinflammatory signals [163–165], which contribute to atherosclerotic plaques susceptible to myocardial infarction and strokes[166]. Indeed, the fact that vasculopathy pervasively occurs even in the oxygenated conditions in both small and larger vessels demands a new understanding of SCD pathophysiology in the absence of vaso-occlusion, which occurs only under the deoxygenated conditions in the microvessels.

Inspired by advances in the mechanistic understanding of the distribution and segregation behaviors during blood flow as well as experimental observations of blood disorders during flow [1–3, 131, 155], we propose a biophysical hypothesis for the pathophysiology of vasculopathy in blood disorders: diseased cells strongly marginate, residing primarily in the CFL near the vascular walls, resulting in endothelial inflammation by provoking fluctuations in local wall shear stress, which is consistent with the chronic and diffusive nature of vasculopathy in blood cell disorders. Limited computational studies of this hypothesis, for blood flow in straight tubes, have been performed for the cases of SCD [167] and IDA [131].

The present work uses detailed simulations of a cellular-scale mathematical model of blood flow in small vessels to examine this hypothesis. Several diseases are considered: SCD, IDA, COVID-19, and spherocytosis, in both a simple cylindrical blood vessel geometry and a more geometrically complex serpentine curved tube. The choice of these disease models arose from a number of considerations. On biophysical grounds, all of these disorders result in subpopulations of red cells with altered physical, morphological, and geometric properties. Biologically, they represent a spectrum of disorders that encompass different etiologies, illustrating the generalizability of our findings: SCD arises from a genetic disorder, iron deficiency anemia a nutritional one; COVID-19 is an example of an infectious disease that gives rise to biophysically altered red cell subpopulations, and spherocytosis can arise in genetic or acquired disorders. Not only do the results provide strong and broad-based computational support for our hypothesis, but they also begin to reveal transient aspects of the stress environment experienced by endothelial cells as well as the strong spatial variations in this environment engendered by a complex flow geometry.

An important and distinctive aspect of the study is its focus on mechanism. Because the study is computational, we can interrogate the results in exquisite detail, revealing for example not only the presence, but also the physical origin of a strong localization of aberrant cells in specific regions near the walls of a complex blood vessel. Furthermore, as a computational study, it can also avoid factors such as the broad variability in cell properties,

which are inevitable in *in vivo* studies and hard to avoid even in carefully designed *in vitro* studies, that can obscure the dominant phenomena.

2.2 Results

2.2.1 Model summary

We simulate a flowing suspension of RBCs, modeled as deformable fluid-filled elastic capsules, in rigid straight and curved cylindrical tubes with diameter $D = 40\mu m$. Unless otherwise stated, all results are for simulations that have been run to a statistically stationary state. For the blood disease cases, RBC suspensions are modeled as binary mixtures of normal RBCs with aberrant RBCs from different blood disorders (e.g., idRBCs, sickle RBCs, spherocytes, and spherocytes). In the binary suspensions, the number fraction for normal RBCs is 0.9, and for aberrant RBCs is 0.1. This is a simplification, as in any real blood cell population there will be a distribution of cell properties. A suspension of only normal RBCs, referred to as healthy RBC suspension, is considered as a control. The overall volume fraction (tube hematocrit) is around 20%, consistent with the observed substantial decrease of hematocrit from large vessel to the microcirculation [168, 169]. (There is some variation between the cases we consider here because different cell types have different volumes – what we keep constant between cases are number fraction and number density.) The suspending fluid, blood plasma, is considered incompressible and Newtonian with a viscosity of about $\eta = 1.10 - 1.35\text{mPas}$. The discoid radius a for human RBC is about $4\mu m$. The RBC membrane in-plane shear elasticity modulus $G \sim 2.5 - 6\mu\text{N/m}$. The deformability of a capsule in the pressure-driven flow is measured by the dimensionless capillary number $Ca = \eta\dot{\gamma}_w a/G$. Ca is set to be 1.0 for normal RBCs, which corresponds to $\dot{\gamma}_w \sim 1000\text{ s}^{-1}$.

Increased membrane stiffness has been identified in a range of blood cell disorders. For example, evidence exists that the membrane shear modulus of a typical sickle cell is approximately four times greater than that of a healthy RBC [124]. For IDA, measurements indicate

the presence of iron deficient RBCs with stiffness up to ten times greater [131]. Subpopulations of RBCs with substantially increased membrane stiffness and smaller dimensions have also been found in COVID-19 [132], and spherocytosis [170]. Consequently, for the present study the interfacial shear modulus G of aberrant cells is taken to be five times that of normal RBCs. Therefore, Ca for the aberrant RBCs in our study is at most 0.2 times that for normal RBCs.

The spontaneous shape of the RBC membrane is inhomogeneous. Dupire et al. [171] showed that an RBC maintains its biconcave shape even during tank-treading and hypothesizes that this effect might come from anisotropic elastic properties or an inhomogeneous natural shape. Fischer et al.[172] found that RBCs have “shape memory”, which arises from spatial variations in their natural shape. The choice of the spontaneous shape can strongly affect the stable dynamics of the RBC. Sinha et al.[173] investigate the cell dynamics’ dependence on the membrane’s spontaneous curvature. They found that an oblate spheroidal spontaneous curvature maintains the dimple of the RBC during tank-treading dynamics and exhibits off-shear-plane, tumbling consistent with the experimental observations of Dupire et al.[171]. For a complex structure such as an RBC membrane, it is possible that the natural shape for shear elasticity may differ from that for bending elasticity so the overall natural shape of an element results from the balance of bending and shear forces. Thus in this work, the spontaneous shape of RBC bending elasticity is taken to be the oblate spheroid, while the spontaneous shape of RBC shear elasticity is assumed to be the biconcave discoid. Further details are included in the Materials and Methods section and Supplementary Information; in particular we show that the results here are robust against changes in the details of the cell elasticity model.

2.2.2 Cylindrical blood vessel

Figures 2.1 (A-D) show snapshots from simulations of blood flow in a straight tube for SCD, IDA, COVID-19, and spherocytosis, respectively. (The SI contains movies of these

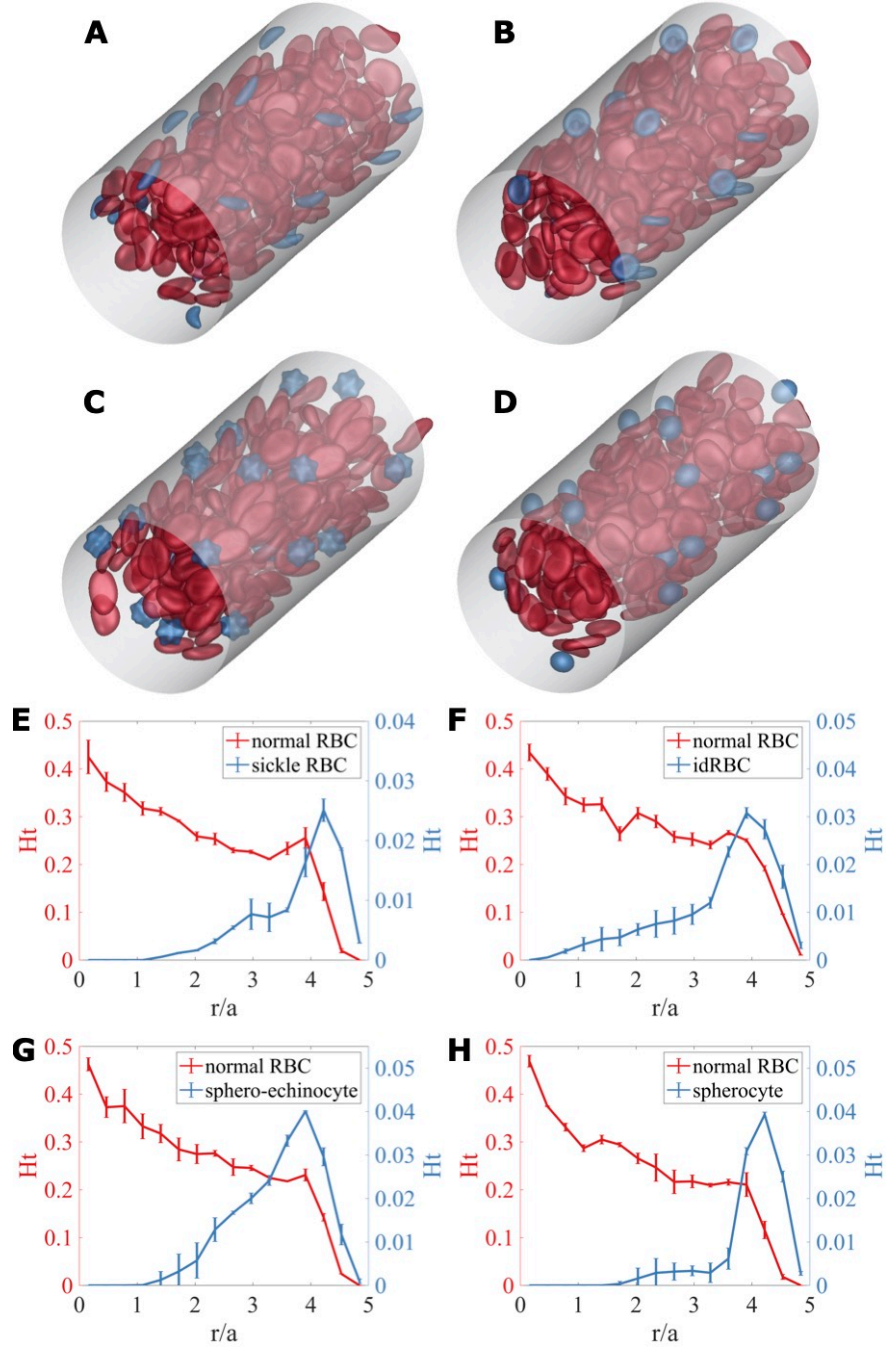


Figure 2.1: Cell distributions within a cylindrical blood vessel. (Top) Simulation snapshots for (A) sickle cell disease, (B) iron deficiency anemia, (C) COVID-19, and (D) spherocytosis, in straight cylindrical tube subjected to a unidirectional pressure-driven flow. Red capsules represent normal RBCs with oblate spheroid shape for bending elasticity and biconcave discoid shape for shear elasticity, while blue capsules are for aberrant RBCs. (Bottom) Radial hematocrit profile for (red) normal RBCs and (blue) aberrant RBCs of (E) sickle cells in SCD, (F) idRBCs in IDA, (G) sphero-echinocytes in COVID-19, and (H) spherocytes in spherocytosis. In all figures, error bars represent estimated standard error using the block averaging method [5].

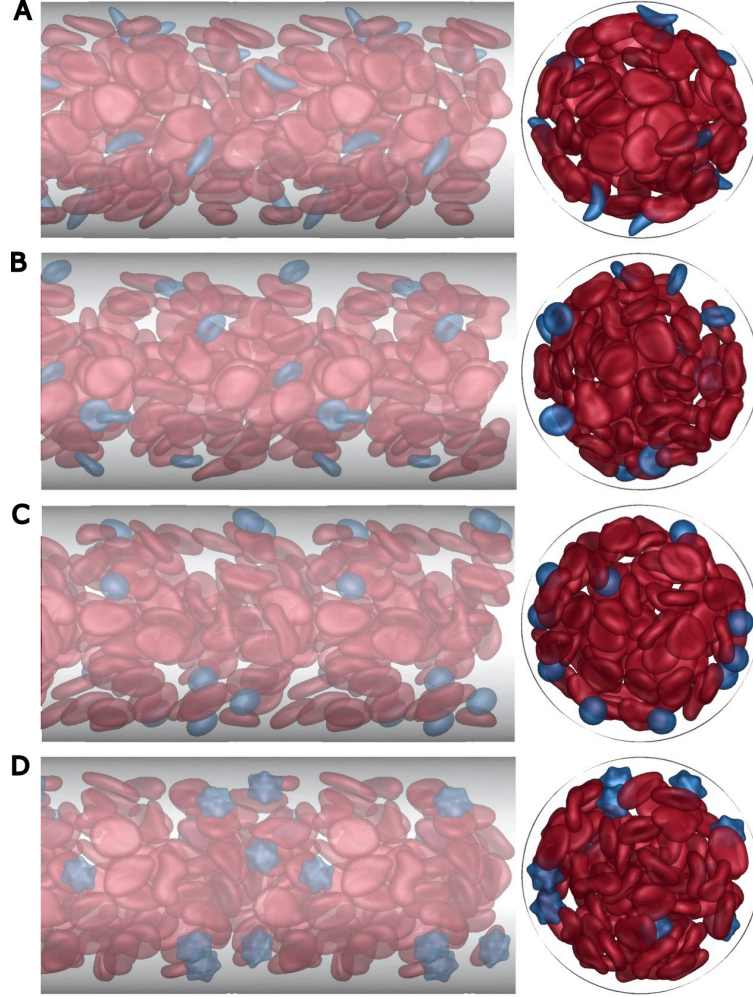


Figure 2.2: Simulation snapshots (left: side view; right: top view) for (A) SCD, (B) IDA, (C) spherocytosis, and, (D) COVID-19 RBC suspension in the cylindrical straight channel.

simulations.) In all cases, the aberrant cells (blue) appear to be margined. Fig.2.1(E-H), respectively, show the corresponding radial hematocrit profiles. These indicate that aberrant RBCs strongly marginate, while the normal RBCs display the expected CFL, and a concentration that increases toward the centerline. Sample simulations with doubled tube length and the same mesh spacing were also conducted; changes in the results were negligible. These results demonstrate that differences in cell size and deformability of the aberrant cells are sufficient to drive strong segregation behavior.

The presence of stiff and/or small aberrant cells near vessel walls is expected to generate high velocity gradients, and consequently large shear stresses on the walls, τ_w . Fig.2.3

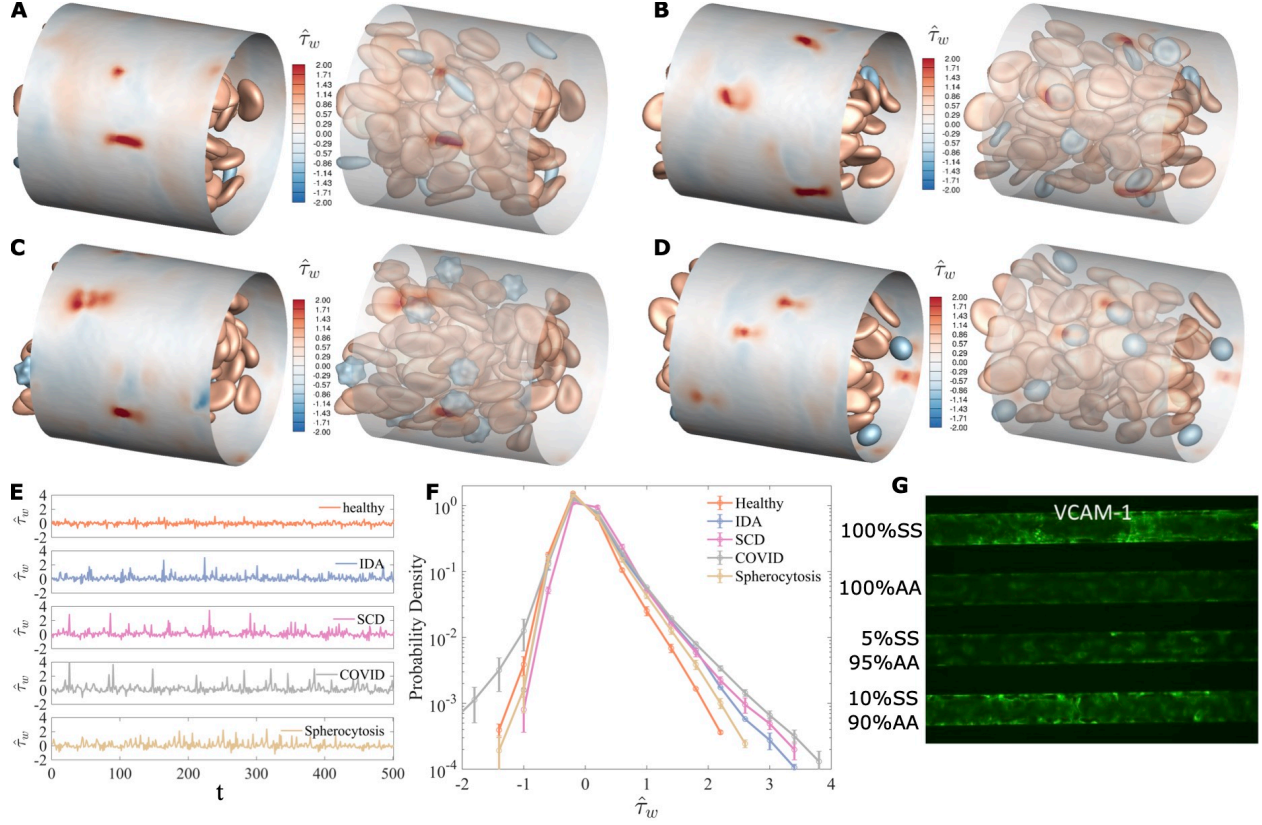


Figure 2.3: Shear stress on a cylindrical blood vessel. (Top) Simulation snapshots and corresponding transparent views of excess wall shear stress $\hat{\tau}_w$, induced by the presence of the cells in (A) sickle cell diseases, (B) iron deficiency anemia, (C) COVID-19, and (D) spherocytosis RBC suspensions. To distinguish the colors of the cells themselves from the colors of the RBC-induced wall shear stress on the cylindrical surface, the color of normal RBCs is set to be pale red, and aberrant RBCs pale blue. (Bottom) (E) Time evolution of the additional wall shear stress $\hat{\tau}_w$ (i. e. deviation from the mean) at a fixed wall position for the cases of a homogeneous suspension of healthy RBCs and binary suspensions of normal RBCs with aberrant RBCs, respectively. (F) Probability distribution of the additional wall shear stresses $\hat{\tau}_w$ over the cylindrical wall in the various cases. (G) Endothelial VCAM-1 expression in "endothelialized" microfluidic devices with a width of $100\mu m$ after perfusion of suspensions with various fractions of (AA) normal and (SS) SCD RBCs [1].

(A, B, C, D) shows snapshots of the spatial distribution of excess wall shear stress $\hat{\tau}_w$ for the four cases. Here $\hat{\tau}_w = \tau_w - \bar{\tau}_w$ is defined as deviation from the mean wall shear stress $\bar{\tau}_w$. The red regions indicate large local fluctuations, and one can see that these are directly associated with nearby aberrant RBCs. Fig.2.3(E) shows time series of additional wall shear stress $\hat{\tau}_w$ at a point on the wall for the various cases. Peaks of high additional wall shear stress are larger and more frequent in all of the disease cases than in the healthy case. These differences are further quantified in Fig.2.3(F), which shows the probability density profiles of excess wall shear stress in the suspensions. The PDFs for all aberrant RBC cases display a long tail at high $\hat{\tau}_w$, where the probability density of high wall shear stress for cases with aberrant RBCs is orders of magnitude higher than for the healthy case. This phenomenon is especially prominent for sphero-echinocytes and sickle RBCs and less pronounced with spherocytes, and is related to their morphology: the spiked surfaces of sphero-echinocytes and sickle RBCs induce high local wall shear stress, while the round spherocytes, though near the wall, roll smoothly without generating substantial excess stress.

The simulation findings here are further compared with previous experimental observations in Fig.2.3 (G). To investigate the role of cellular interaction in hematological diseases, Caruso et al. [1] developed an *in vitro* microvasculature model comprised of endothelial cells cultured through the inner surface of a microfluidic system. SCD RBCs were spiked into normal RBC suspension, suspended, and then perfused into this endothelialized microfluidics. They found, VCAM1, a biomarker of endothelial cell dysfunction, was upregulated when exposed to flowing SCD RBCs than normal RBCs. These results together imply that purely physical interactions between endothelial cells and SCD RBCs are sufficient to cause endothelial inflammation.

Moreover, recent research further corroborates our current observations. Specifically, in the context of diabetes-associated oxidative stress leading to reduced RBC deformability, Czaja et al. [174] employed simulations to investigate pulsatile blood flow through segmented retinal microaneurysms. Their findings revealed that diabetic RBCs, characterized

by increased stiffness, induced higher local wall stress and wall shear stress gradients within leading and draining parental vessels, compared to their healthy RBC counterparts. Additionally, leveraging a high-fidelity computational model of blood flow, Ebrahimi et al. [175] revealed that reduced cell deformability causes substantial changes in microvascular hemodynamics, and alteration in RBC dynamics induces localized changes in wall shear stress within vessels and in proximity to vascular bifurcations. However, these investigations, while addressing the influence of blood cell deformability on hemodynamics and wall shear stress, did not incorporate the RBC margination driven by deformability difference as a potential contributor to vascular wall stress fluctuations.

2.2.3 Curved blood vessel

We now consider cell and stress distributions in a curved tube. Fig.2.4 presents simulation results for a suspension of normal RBCs with sickle RBCs. Snapshots of cell distributions are shown in Fig.2.4(*A, B*), along with a coordinate system we use for the analysis. Fig.2.4(*C, E*), show cell number density distributions for the (*C*) normal and (*E*) aberrant cells on the center plane of the channel. The margination of the aberrant cells is apparent. Fig.2.4(*D, F*) show the number density distributions for the normal and aberrant cells averaged over various segments of the channel, including both the normalized center-plane and cross-sectional cell distributions. Fig.2.4(*D*) shows that the CFL thickness is larger near the outer side ($\theta = 0^\circ$) and thinnest near the inner side ($\theta = 180^\circ$). Fig.2.4(*F*) indicates that sickle RBCs strongly focus at two near-wall locations, both at the inner and outer sides, on the centerplane. As the angle ϕ increases (i.e. as we move downstream around a bend), the concentration of sickle cells near the outer wall becomes more pronounced. Similar results are found for the other aberrant cell suspensions as well, as seen in the cross-sectional and centerplane distributions shown in Fig.S11 in SI.

These results demonstrate that in the curved tube, not only do we see margination of aberrant cells as found in the straight tube, but very strong localization of the margined

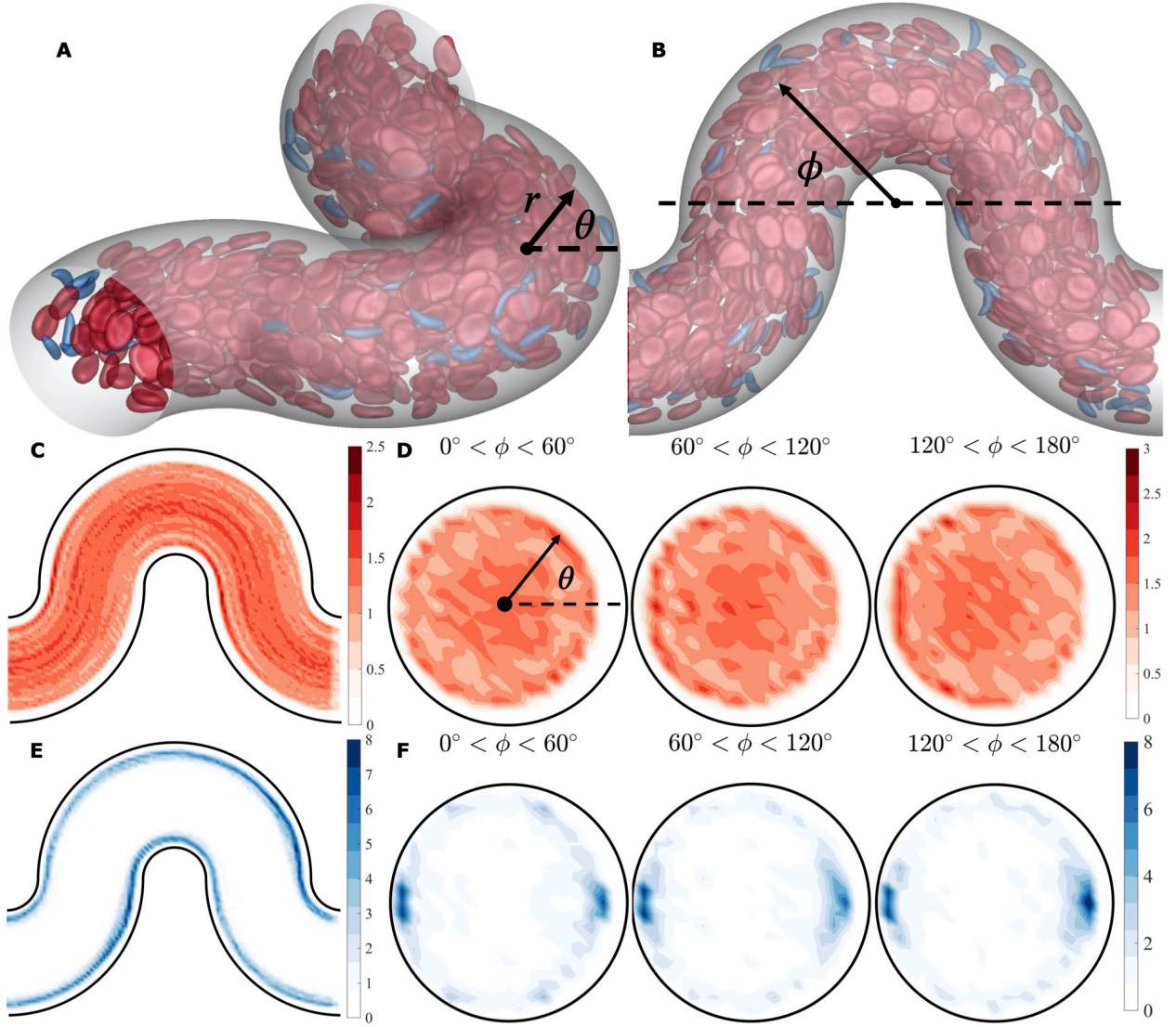


Figure 2.4: Cell distributions within a curved blood vessel. (A, B) Simulation snapshots of a suspension of normal RBCs with sickle RBCs in a curved channel, showing coordinate definitions for r the distance from the local channel centerline, θ , the relative position between the “inner side” ($\theta = 180^\circ$) and “outer side” ($\theta = 0^\circ$), and ϕ , the angle around the curve. (C) Normalized center-plane cell number density and (D) normalized cross-sectional cell number density for normal RBCs in the binary SCD RBC suspension. (E) Normalized center-plane cell number density and (F) normalized cross-sectional cell number density for sickle RBCs in the binary SCD RBC suspension. Note that the curved channel is divided into three parts based on the value of angle ϕ ; thus, the cross-sectional cell number distribution is computed over each part. The cell number density distributions are normalized so that if the spatial distributions of cells is uniform, then the normalized cell number density is unity everywhere within the curved channel.

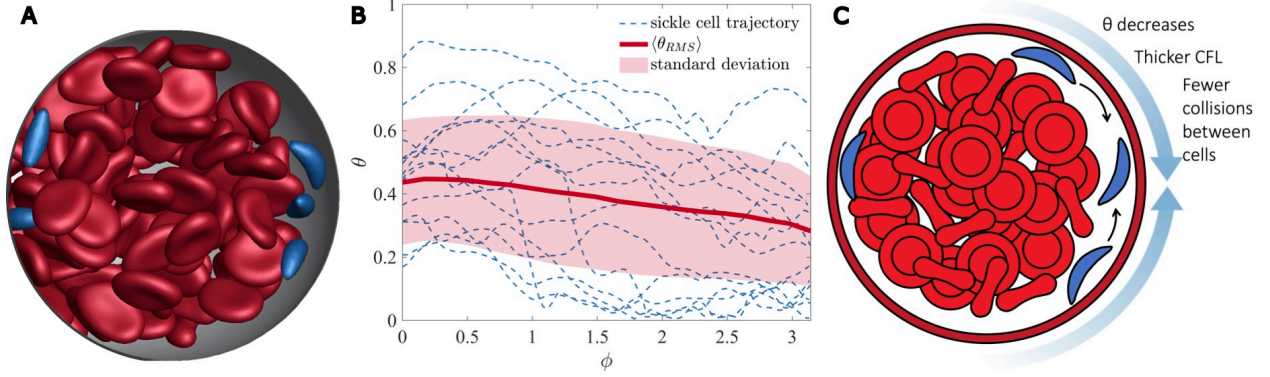


Figure 2.5: Mechanism of cell-localization on the symmetry plane of a curved vessel. (A) Simulation snapshot showing the cross-section cell distribution ($\phi = \pi/2$). (B) Trajectories of sickle RBCs on the $\theta - \phi$ plane. Dashed blue curves are individual trajectories; the red line denotes the root mean squared trajectory of the blue curves, and the red shaded area is for the corresponding standard deviation. (C) A schematic mechanism for localization of margined cells to $\theta = 0$.

cells on the centerplane. The mechanism of this localization originates in the θ -dependence of the cell-free layer thickness, as illustrated in Fig. 2.5. Fig.2.5(A) shows a simulation snapshot of cross-section cell distribution at $\phi = \pi/2$, in which aberrant cells are highly localized near the outer side centerplane. We noted above that the CFL thickness is approximately uniform for $\pi/2 < \theta < 3\pi/2$ – i.e. along the inner wall, but on the outer side, the CFL thickness increases, reaching a maximum on the centerplane on the outer wall – i.e. the CFL thickness increases as $\theta \rightarrow 0$. A margined cell on the outer wall will experience more collisions from the side with the thinner CFL than the thicker, thus being driven on average toward the region where the CFL is thickest, $\theta = 0$. Fig.2.5(B) shows the trajectories of margined aberrant RBCs on the $\theta - \phi$ plane, demonstrating that as ϕ increases, θ tends to decrease and aberrant cells move towards the centerplane. We illustrate this mechanism schematically in Fig.2.5(C). Finally, we must address why there is localization along the centerplane on the inner wall ($\theta = \pi$). This results from the simple fact that the outer wall over half a wavelength of the curved shape is the inner wall over the other half; the CFL thickness is nearly constant along the inner wall, driving no net motion in θ , and cells driven toward $\theta = 0$ on the outer wall tend to remain there while moving along the inner wall.

Fig.2.6(A) shows snapshots of the spatial distribution of excess wall shear stress $\hat{\tau}_w$ in

SCD suspensions; results for the other blood disorders can be found in Fig.S12, S13, S14 in SI. The presence of a sickle RBC close to the wall directly causes local fluctuations in wall shear stress, as can be observed from the transparent view in Fig.2.6(B).

To capture the spatial dependence of RBC-induced wall shear stress $\hat{\tau}_w$, the probability density profiles of τ_w in SCD and healthy RBC suspensions over the different θ -areas on the vascular surface are presented in Fig.2.6(C). In the healthy RBC suspension, the probability of high $\hat{\tau}_w$ is largest near the inner side ($3\pi/4 < \theta < \pi$), followed by the intermediate area of ($\pi/4 < \theta < 3\pi/4$), and smallest over the outer side ($0 < \theta < \pi/4$), consistent with the fact that the CFL is thinnest near the inner side and thickest near the outer side. As in the straight tube, it is observed that the cases with aberrant RBCs exhibit a distinct excess of large positive fluctuations, again attributable to the margination of these small stiff cells to the vessel wall. Furthermore, the disparity at high RBC-induced wall shear stress $\hat{\tau}_w$ of the probability distribution profiles between diseased and healthy RBC suspensions is most pronounced at the outer side, which implies that the localization of the marginated aberrant RBCs to the center plane elevates the probability of high additional wall shear stress by an order of magnitude over the outer side wall of the curved tube.

The geometries of blood vessels have been found to play a role in the development of endothelial dysfunction in blood disorders. Using an endothelialized microfluidic model, Wang et al.[3] discovered that endothelial cells exposed to SCD RBCs exhibited an increase in endothelial inflammation along the outside wall of the bend in the curved regions of vessels. This result is illustrated in Fig.2.6(D), which shows expression levels of E-selectin, which is upregulated when cells are in a pro-inflammatory state, upstream and in a curved segment of endothelialized microfluidic channels through which RBC suspensions have flowed. While the channel sizes in the experiments are much larger than those simulated here, the qualitative pattern of endothelial inflammation there is consistent with the margination patterns we observe here.

To further quantify the cellular segregation in different geometries, we measure the scaled

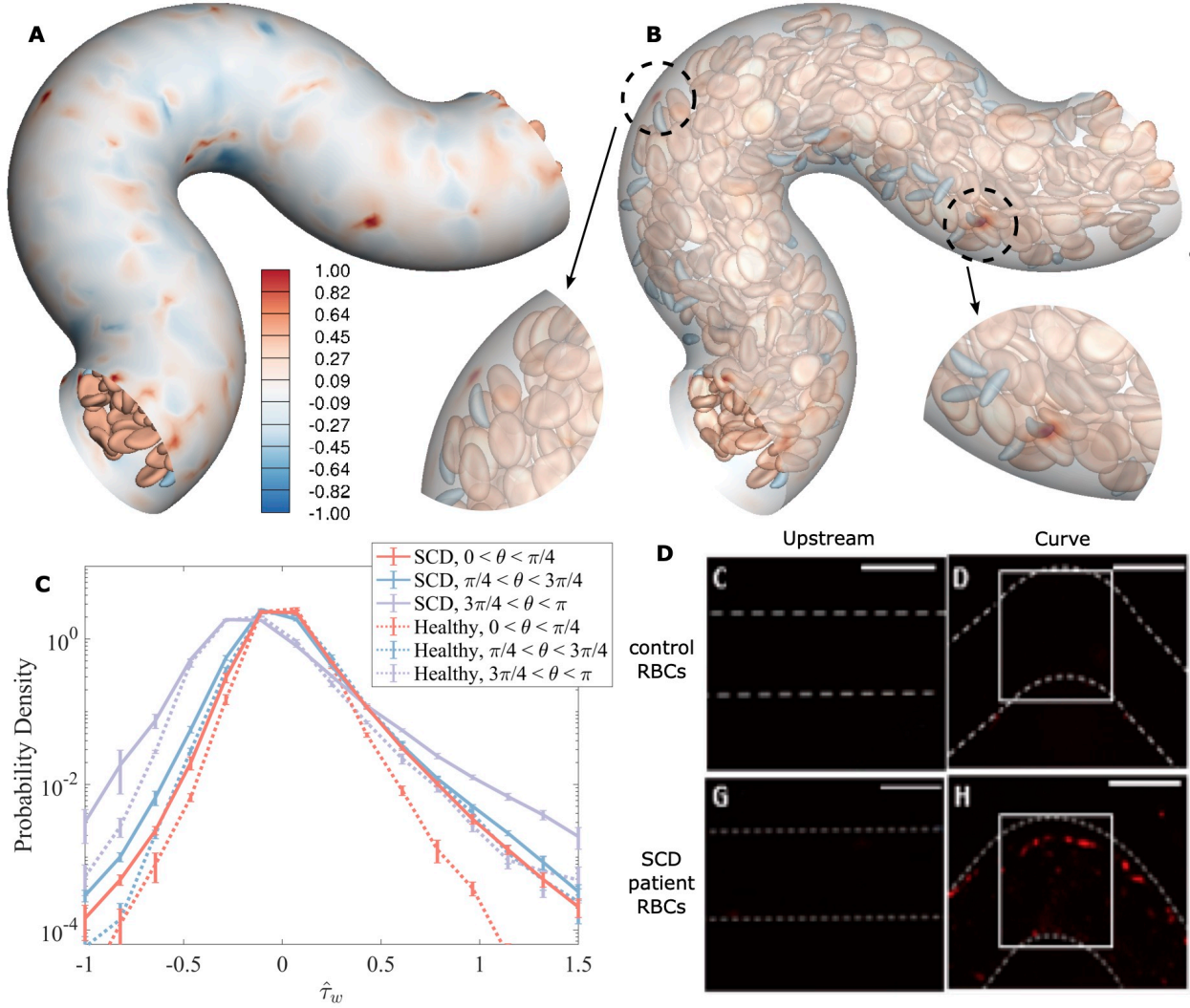


Figure 2.6: Shear stress on a curved blood vessel. (A) Simulation snapshots and (B) corresponding transparent views of additional wall shear stress $\hat{\tau}_w$ induced by the presence of the cells in suspensions of normal RBCs with sickle RBCs within the curved channel. The color on the vascular surface denotes the RBC-induced wall shear stress strength $\hat{\tau}_w$. (C) The time-averaged probability distribution of the additional wall shear stress $\hat{\tau}_w$ for SCD RBC suspension and healthy RBC suspension over the different θ -area on the curved vascular surface. (D) Mechanically stiff SCD RBCs upregulate E-selectin at the curvature site of vasculature models, indicating pro-inflammatory endothelial signaling [3]. Scale bars = $200\mu m$

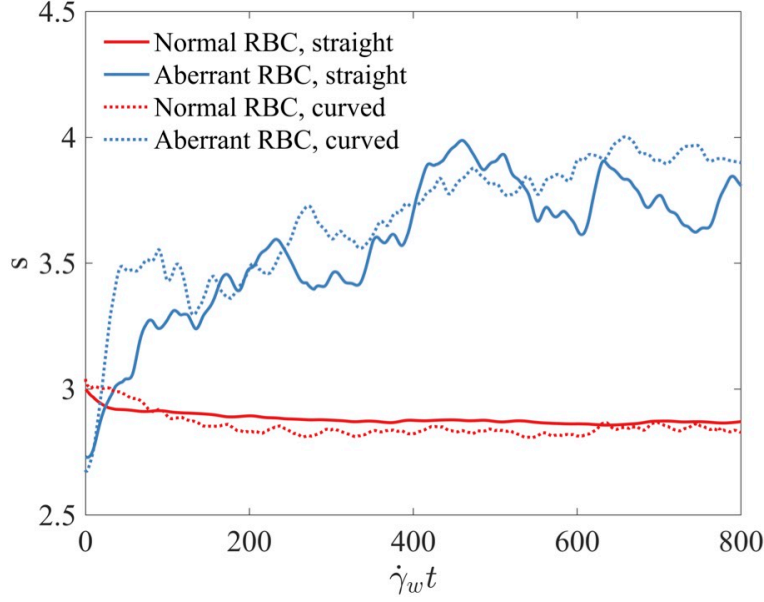


Figure 2.7: Segregation in straight and curved channels. Temporal evolution of RMS distance from the centerline, $s = \langle r_{cm}^2 \rangle^{1/2} / a$ for each cellular component (red, normal RBCs; blue, aberrant RBCs) in an SCD RBC suspension within the straight tube (solid line) and curved channel (dash line). r_{cm} is the radial center-of-mass position of a cell and angle brackets denote averaging over the cells in the system. Both the cylindrical straight and curved channels have a tube radius of $20\mu m$.

root-mean-square (RMS) distance from the centerline, $s = \langle r_{cm}^2 \rangle^{1/2} / a$, for each cellular component in straight and curved channels. At the start of the simulations, all cells are randomly distributed within the vascular channels. Fig.2.7 illustrates the temporal evolution of s for both normal and sickle RBCs. Initially, the aberrant RBCs show a sharp increase in s , then plateau with $s \approx 3.8$; in contrast, for normal cells $s \approx 2.9$. This behavior is similar in both straight and curved channels, consistent with the above observations that aberrant cells are localized to vessel walls.

Here we have measured time in terms of strain units $\dot{\gamma}_w t$, and Fig.2.7 indicates that, for margination under the present conditions, ~ 200 strain units are required for margination. This can be translated roughly into downstream distance by noting that the mean velocity for laminar flow with wall shear rate $\dot{\gamma}_w$ is $\dot{\gamma}_w R$. Thus in the time of about 200 strain units required for margination to take place, a cell moving at the mean velocity travels 2mm.

Additionally, we observe that segregation occurs somewhat more rapidly in the curved

channel compared to the straight channel, as indicated by the faster growth of s for aberrant cells after the simulation starts. While we are unaware of other work on margination in serpentine geometries, relatedly, Bächer et al. [176] explored the margination of microparticles in blood flow through bifurcations within vascular networks. They found that bifurcations did not substantially influence the margination propensity of stiff particles relative to flow in straight tubes.

2.3 Discussion

Blood disorders lead to changes in red blood cell size, shape, and stiffness, and thus to changes in how aberrant cells are distributed in the cross-section of blood vessels, and changes in the interaction between cells and blood vessels. Inflammation and dysfunction of endothelial cells lining blood vessels are associated with the risk of pathophysiologic complications like stroke and atherosclerosis.

This study describes results from detailed cell-level simulations of blood in straight and serpentine tubes, addressing the hypothesis that the margination of aberrant cells leads to substantial changes in the local shear stress environment of the blood vessel wall, possibly contributing to the observed dysfunction and inflammation. We compare cell distributions and wall shear stress profiles between suspensions of normal blood and blood containing aberrant RBCs that model sickle cell disease (SCD), iron deficiency anemia, COVID-19, and spherocytosis. In all cases, the smaller and stiffer aberrant RBCs marginate and increase the fluctuations in wall shear stress. Probability density profiles of wall shear stress show that cases with aberrant RBCs display a significantly higher probability of high wall shear stress than in suspensions of healthy cells. The difference is most notable in COVID-19 and SCD RBC suspensions.

In the serpentine curved tube case, the margined aberrant cells tend to marginate most strongly to the symmetry plane of the channel and the outer side of the curved tube, becom-

ing very strongly localized in those regions. This result implies the possibility of strongly localized endothelial damage in blood vessels with complex geometries. These findings highlight the importance of considering vascular geometry and the presence of aberrant RBCs in the development of vasculopathy.

Overall, our study indicates that the biophysical alterations of red cells in various disorders, in and of themselves, can directly alter the shear stress the underlying endothelium is exposed to. This suggests that that red cell biophysics and the pathologic changes thereof may directly affect endothelial mechanobiological pathways, which, in turn, may be associated with chronic endothelial inflammation or dysfunction that lead to disorders such as cardiovascular disease and stroke.

Experiments to complement these computational results are now being conducted in our group. In addition, our study also suggests that clinically, more attention should be paid to the biophysical alterations of the red cells themselves, which currently are viewed as hallmarks of the associated disease but not necessarily as biomarkers per se; our work suggests otherwise and indicates that the red cell shape, size, deformability should be re-examined more rigorously as potential correlates or predictors of clinical endpoints. Finally, our work indicates that therapeutic interventions that “fix” or remove the biophysical alterations of red cells should be explored for the associated diseases. Indeed red cell pheresis for various hematology conditions may improve the vascular dysfunction associated with those diseases and for sickle cell disease in particular, recently FDA-approved treatments that improve “red cell health” may also alleviate vasculopathy.

2.4 Materials and Methods

We consider a flowing suspension of RBCs, which we model as deformable fluid-filled elastic capsules, in rigid straight (Fig.2.1) and curved “serpentine” (Fig.2.4) cylindrical tubes with radius $R = 20\mu\text{m}$. No-slip boundary conditions are imposed on the walls of the tube,

while periodic boundary conditions are applied in the flow direction. The suspension is subjected to a unidirectional pressure-driven flow, and the velocity field in the absence of RBCs field within the straight cylindrical tube is given by the Poiseuille flow. In this study, the flow is driven by a constant pressure gradient, which is equivalent to fixing the mean wall shear rate at $\dot{\gamma}_w = 2U_0/R$, where U_0 is the undisturbed centerline velocity. For the curved cylindrical channel, the pressure gradients are determined using defining an equivalent straight cylindrical channel with the same centerline length and radius.

This study considers both homogeneous and heterogeneous suspensions of various components, including normal RBCs and aberrant RBCs (e.g., iron deficiency RBCs, sickle RBCs, sphero-echinocytes, and spherocytes). In binary suspensions, normal RBCs are generally considered primary components (denoted as “p”), while aberrant RBCs as trace components (denoted as “t”). A normal RBC is modeled as a flexible capsule having the spontaneous shape being a biconcave discoidal for shear elasticity and an oblate spheroid for bending elasticity [173, 177], with a radius of $a = 4\mu m$. The idRBCs have the same rest shape as normal RBCs, except the radius of idRBCs is $0.76a$ [131]. The rest shapes of sickle RBCs, spherocytes, and sphero-echinocytes are curved oblate, spherical with a diameter of $5\mu m$, and spiked spherical, respectively. The cell membranes are modeled as an isotropic and hyperelastic surface with interfacial shear modulus G , incorporating shear elasticity, area dilatation, volume conservation, and bending resistance. Details of the membrane mechanics model and validation against experimental observations are given in [173].

The deformability of a capsule in pressure-driven flow is measured by the dimensionless capillary number $Ca = \eta\dot{\gamma}_w a/G$. In this study, G of the aberrant RBCs is assumed to be five times that of normal RBCs, which leads to that Ca_t for aberrant RBCs is always around less 0.2 times that of Ca_p for biconcave discoid RBCs. In this study, Ca_p is set to 1.0 for normal RBCs, Ca_t is 0.15 for idRBCs, 0.20 for sickle RBCs, 0.15 for sphero-echinocytes, and 0.125 for spherocytes, which corresponds to $\dot{\gamma}_w \sim 1000 \text{ s}^{-1}$. In the binary suspension, the number fractions for normal RBCs X_b is set to 0.9, and for aberrant RBCs, X_t is 0.1, so the overall

number density ratio $n_p/n_t = 9$. In this study, the total cell volume fraction (hematocrit) is set to be $\phi \approx 0.20$. To simplify the computations in this initial study, the suspending fluid and the fluid inside the cells are assumed to have the same viscosity.

In our simulation, the particle Reynolds number, defined as $Re_p = \rho \dot{\gamma}_w a^2 / \eta$, is set to be 0.1, and the fluid is assumed to be incompressible and Newtonian; therefore the flow is governed by the Navier-Stokes and continuity equations. A projection method is used to advance the velocity field in time. The straight tube is embedded in a cuboidal computational domain with the size of $10a \times 10a \times 10a$, and an Eulerian grid of $100 \times 100 \times 100$ is used. For the serpentine channel case, the cuboidal computational domain's size is $32a \times 26a \times 10a$, and the Eulerian grid of $320 \times 260 \times 100$ is used. The immersed boundary method (IBM) is used to handle fluid-structure interaction. Specifically, the current model considers two types of immersed boundaries: deformable moving cellular membranes and rigid nonmoving vascular walls. The capsule membrane is discretized into N_Δ piecewise flat triangular elements; $N_{\Delta p} = 1280$ for normal RBC, while $N_{\Delta t} = 682$ for sickle RBC, $N_{\Delta t} = 816$ for idRBC, $N_{\Delta t} = 620$ for spherocytes, and $N_{\Delta t} = 1134$ for sphero-echinocytes. Different N_Δ are chosen to ensure that the triangular elements on both capsules are close in size. We use “continuous forcing” IBM and “direct forcing” IBM methods for the RBC membranes and tube wall, respectively. The numerical methodology follows the approach described in [178, 179].

Several limitations pertain to the model we employed in this study. Firstly, we assume a viscosity ratio of one between the intercellular matrix and plasma, whereas normal RBCs may exhibit a ratio of up to fifteen [180], and aberrant RBCs may possess even higher ratios. As the RBC ages, the viscosity of the cytosol increases [181]. Nonetheless, past research [136] has demonstrated that the dynamics of a single sickle cell remain qualitatively unchanged over a broad range of viscosity ratio. Additionally, Reasor et al. [182] performed simulations to investigate the margination dependence on the viscosity ratio, finding that the cell-free-layer thickness and margination of stiff cellular components remain similar. Another limitation of this study is the assumption of uniform shape, size, and stiffness within each red cell

subpopulation, which we know does not fully account for the inherent diversity in these properties present in real red cell populations [183, 184]. Nevertheless, while the physical properties of blood cells exhibit dispersity, the variation of these properties within one cell subpopulation is substantially narrower when compared to the variation between different cell subpopulations (see e.g. [131]). Thus we expect that this simplification does not alter the main conclusions drawn here.

Furthermore, sickle cells display increased adhesiveness to the vascular wall, triggering the vaso-occlusion that is the hallmark of SCD. Claveria et al. [155] underscore the role of cell aggregation in mitigating segregation and amplifying CFL thickness, potentially guarding against vaso-occlusion in sickle cell anemia patients. In the present work, we do not consider intercellular forces such as aggregation and adhesion, for two reasons. First, our focus is the broader issue of the role of margination across blood disorders, not just sickle cell disease. Furthermore, aggregation effects are most prominent at low shear rates, $\lesssim 10s^{-1}$, whereas the wall shear rate $\dot{\gamma}_w$ in our simulation remains substantially higher, $\sim 1000s^{-1}$, a condition representative of venules and arterioles.

Finally, endothelial cells exposed to shear stress express an endothelial glycocalyx layer (EGL) that plays a central role in mediating vascular permeability and endothelial anti-inflammatory, antithrombotic, and antiangiogenic properties [46]. Notably, EGL disruption occurs in arterial regions exposed to disturbed blood flow, accompanied by elevated oxidative stress leading to atherogenesis [185, 186]. Our model, which treats the vascular surface as a non-slip rigid boundary, does not capture the permeability and porosity characteristics of EGL. However, it is important to underscore that the presence of EGL, while affecting interactions of RBC with vessel walls, is not anticipated to significantly impact the margination of aberrant cells, which primarily arises from contrasting cellular properties between aberrant and normal RBCs. In addition, our study focuses on conditions within venules and arterioles, where vessel-cell interactions are less pronounced compared to the confined spaces of capillaries. Therefore, the limitations of our model do not change the central findings in

this research: the aberrant cells strongly marginate, residing primarily in the cell-free layer, and generating physical interactions that damage the endothelium.

3

Microcirculatory blood flow with aberrant levels of red blood cell aggregation ¹

¹The text of this chapter is adapted from the publication by X. Cheng, D. Zimmerman, E. Iffrig, W. A. Lam, and M. D. Graham. *arXiv*, 2411.18703

Recent clinical results indicate that aberrant erythrocyte aggregation in hematological disorders is accompanied by endothelial damage and glycocalyx disruption, but the underlying biophysical mechanisms remain unclear. This study uses direct computational modeling to explore how red blood cell (RBC) aggregation impacts shear stress in small blood vessels, highlighting the increased risk of vascular damage. RBC aggregation creates a heterogeneous distribution, leading to variations in the cell-free layer thickness and fluctuating wall shear stress, especially near vessel walls. This effect aligns with experimental findings on endothelial disruption linked to RBC clustering near the wall, potentially reducing the protective glycocalyx layer. The power spectral density analysis of wall shear stress fluctuations reveals that, with RBC aggregation, there is a distinct peak near frequency $f = 0.04$, indicating increased fluctuations due to aggregated RBC clusters traveling close to the vessel wall. The presence of aberrant cells in blood disorders, modeled here by sickle cells, further amplifies these effects, as aggregation-enhanced margination drives sickle cells closer to vessel walls, exacerbating shear stress fluctuations and increasing the likelihood of vascular injury and inflammation. Simulations show that curved vascular geometry, with curvature accentuating RBC clustering near vessel walls, intensifies aggregation-induced wall shear stress fluctuations and increases the risk of vascular damage, particularly in sickle cell disease where sickle cells marginate closer to the wall.

3.1 Introduction and background

The tendency of red blood cells to aggregate plays a significant role in blood flow and circulation. The primary cause of RBC aggregation is the interaction between certain proteins on the surface of red blood cells and various plasma proteins, particularly fibrinogen. Fibrinogen, a glycoprotein found in the plasma, acts as a bridge between adjacent red blood cells, promoting their adhesion and aggregation. Other factors, such as cell surface charges and the presence of certain substances in the plasma, also contribute to the degree of ag-

gregation. While some degree of red blood cell aggregation is normal, aberrant aggregation levels can have implications for blood flow and cardiovascular health. An important effect that is observed in a variety of disorders is that endothelial cells lining the blood vessels are dysfunctional and in a pro-inflammatory state. This is associated, for example in COVID, sepsis, and sickle cell disease, with substantially increased risk of stroke and other serious circulatory problems [118, 145–151]. Very recently, using a microfluidic microvasculature model, Druzak et al. [118] established for the first time that flow of blood with a high level of fibrinogen (and thus propensity to aggregate) directly damages the glycocalyx, the layer of glycoproteins covering cell membranes of endothelial cells. Understanding the mechanisms and factors influencing red blood cell aggregation is essential for gaining insights into vascular health and developing strategies for managing conditions related to blood flow and circulation. This study uses cell-level simulations to investigate erythrocyte aggregation in the microcirculation and its interactions with the vessel wall, considering factors such as blood disorders, flow conditions, and cellular physical properties.

3.1.1 Response of the vascular endothelium to stress

Endothelial cells mechanotransduce the shear forces of the hemodynamic microenvironment into cellular biological signals [163, 166, 187]. Bao *et al.* [188] measured the mRNA expression of MCP-1 and PDGF-A, two genes related to endothelial inflammation, by endothelial cells subjected to laminar flows with different well-defined wall shear stress profiles, namely (1) ramp flow in which shear stress was smoothly changed from zero to a maximum value and then sustained, (2) step flow in which shear stress was abruptly applied at flow onset followed by steady shear stress for a sustained period, and (3) impulse flow in which shear stress was abruptly applied and then removed. A steady shear stress profile was also considered for comparison. They showed that rapid changes in shear stress and steady shear stress, respectively, stimulate and diminish the expression of both genes. In particular, the impulse flow case was found to induce the highest level of endothelial expression of the pro-

inflammatory signals. Other studies [163, 189–197] have also elucidated a variety of shear stress-induced signal transduction pathways of the endothelium, and revealed the opposite effects of steady and unsteady shear stresses on endothelial dysfunction.

To investigate the role of cellular interaction with the vascular endothelium in hematological diseases, Caruso et al. [1] developed an *in vitro* microvasculature model comprised of endothelial cells cultured on the inner surface of a microfluidic system. Sick cell disease (SCD) RBCs were spiked into normal RBC suspension, suspended, and then perfused into this endothelialized microfluidic. They found that VCAM1, a biomarker of endothelial cell dysfunction, was upregulated when exposed to flowing SCD RBCs. These results together imply that purely physical interactions between endothelial cells and SCD RBCs are sufficient to cause endothelial inflammation. A similar study was performed to model iron deficiency anemia (IDA) [198]. IDA suffers have a subpopulation of RBCs that are smaller and stiffer than healthy RBCs, and this study found, as with SCD, that perfusion of mixtures of healthy and iron-deficient RBCs leads to upregulation of markers of pro-inflammatory response. Furthermore, theoretical and computational studies indicate that diseased cells are likely to marginate to blood vessels walls because of their smaller size and higher stiffness [159, 199, 200], and that margination leads to increased frequency of events of high wall shear stress [167, 198, 201, 202]. These observations indicate that margined aberrant cells may play a substantial role in the vasculopathy observed in many hematological disorders.

3.1.2 Aggregation in blood flow: experimental and computational observations

Aggregation of RBCs at rest and low shear rates is a well-known and well-studied phenomenon. Microstructurally, it leads, in combination with the discoidal shape of RBCs to the formation of stacks of RBCs known as rouleaux. Rheologically, it leads to a small yield stress and a regime at low shear rates ($\lesssim 100s^{-1}$) of substantial shear-thinning and thixotropy [203].

Mechanistically, aggregation of RBCs is generally attributed to the presence of fibrinogen, which binds to RBCs membranes leading to aggregation by a bridging mechanism. However, classical depletion flocculation (which is osmotic in origin [204]) due to the presence of the large number of albumin and globulin molecules also plays some role [205, 206]. Indeed, aggregation is frequently induced in experiments with blood via the depletion mechanism by addition of dextran. At the level of modeling undertaken in the present work, we will not need to distinguish between the two mechanisms, simply using a phenomenological potential to describe aggregation forces between points on neighboring cells. We detail the approach in Section 3.2.

One natural question regarding aggregation is the effects it might have on how cells are distributed in the microcirculation (the blood vessels $< 100\mu m$ in diameter) during flow. Key features of this distribution are the presence of (1) the cell-free layer (CFL), and (2) the phenomenon of margination in which white cells and platelets (and also aberrant cells, as noted above) are preferentially found near blood vessel walls, i.e. in the CFL. The basic mechanism of these phenomena in terms of cell-cell collision dynamics and wall-induced migration is understood [159, 199]. Regarding the cell-free layer, one might expect that this is thickened by aggregation as the RBCs tend to pack together under attractive forces. Several experimental studies have confirmed this intuition (as do simulation results below). Ong et al. [207] investigated the impact of red blood cell aggregation and flow rate on the arteriolar cell-free layer in the rat cremaster muscle. By inducing aggregation with dextran 500 infusion, they observed enhanced cell-free layer formation in arterioles. Interestingly, the same group [208] also later found that the *variance* of the CFL thickness also increased under aberrant aggregation conditions – we also see this in our results below. Similar experimental observations were made using a microchannel with a T-junction [209].

Claveria et al [155] studied the distribution and margination of sickle red blood cells (sRBCs). In nonaggregating suspending fluid, low-density (more flexible) sRBC stayed at the channel center, while the densest (least flexible) cells segregated towards the walls. Our

prior computational studies, described in refs. [167, 202], capture this effect. As in the studies noted above, aggregation led to an increase in CFL thickness, while also inhibiting segregation. The latter is a somewhat counterintuitive result, as a thicker CFL leaves more room near blood vessel walls for stiff or small cells to reside, promoting margination. Again, the computational results below are consistent with these experimental observations.

In COVID, fibrinogen levels can be several times normal, resulting not only in increased aggregation (hypercoagulability) but increased plasma viscosity (hyperviscosity), again up to several times normal [210]. Thus the overall blood viscosity is also much higher than normal, despite the hematocrit generally being lower [211]. This is a distinctive characteristic of COVID; in other hyperviscosity syndromes high viscosity comes from immunoglobulin association, not fibrinogen.

Recent advances have provided valuable insights into erythrocyte aggregation. Dynar et al. [212] investigated platelet margination under varying RBC aggregation energies using 2D simulations. Their findings indicate that moderate RBC aggregation enhances platelet margination in microcirculation, whereas excessive aggregation leads to a decline in margination. Using a combination of experiments and simulations, Dasanna et al. [213] examined the dependence of RBC sedimentation rates on fibrinogen concentration and its relationship to the microstructure of gel-like erythrocyte suspensions. Their results demonstrated that higher fibrinogen concentrations strengthen intercellular interactions, creating larger voids in the gel structure, which in turn increases permeability and accelerates sedimentation. Furthermore, recent research [214] revealed that aggregate dissociation rates increase sharply upon reaching a critical extension rate, which falls within the range of microcirculatory conditions. This suggests significant variability in aggregate sizes in vivo.

Many computational studies have addressed the role of RBC aggregation in various aspects of blood flow. Liu et al. [140, 215] proposed a 3D model to explore RBC aggregation and its impact on blood rheology. Their approach effectively captures three significant phenomena in blood rheology: shear rate-dependent viscosity, the influence of cell rigidity on

viscosity, and the Fahraeus-Lindqvist effect. Bagchi et al. [216] presented a 2D simulation of RBC aggregation using an immersed boundary method, considering rheological properties and cell-cell adhesion kinetics. Their results show that higher cytoplasmic viscosity and membrane rigidity lead to stable aggregate formation. Zhang et al. [217] used an immersed boundary lattice Boltzmann algorithm to simulate RBC behavior in shear flows. In 2D shear flows, RBCs aggregate into rouleau-like structures influenced by interaction strength, exhibiting concave shapes in weak interactions and convex shapes in strong interactions. These aggregates rotate or separate in shear flows, aligning with experimental observations. Fedosov et al. [218] accurately predict blood viscosity dependence on shear rate and hematocrit. Xu et al. [219] examined the flow of 49,512 RBCs at a 45% concentration under aggregating forces. Results align well with experimental findings, revealing uniform RBC distributions at moderate shear rates ($60 - 100/\text{s}$) and significant aggregation structures at a lower shear rate $\sim 10/\text{s}$. Yoon et al. [220] developed a method for modeling inter-cellular interactions among multiple adjacent RBCs, analytically determining interaction energy. This approach differs from previous curve-fitting methods for aggregation, enabling predictions of various effects of physical parameters on depletion and electrostatic energy among RBCs. Deng et al. [221] used patient-specific computational simulations to explore fibrinogen-dependent aggregation dynamics in T2DM RBCs, integrating in vitro experiments. The study sheds light on complex cell-cell interactions, emphasizing the significance of RBC aggregation and disaggregation dynamics in patients at a higher risk of microvascular complications.

Despite the substantial number of computational studies of RBC aggregation, they do not consider disease states, and accordingly none have focused on the key issues that we address in the present work: (1) abnormally high levels of aggregation, which will affect blood flow at high strain rates, (2) dynamic and spatially inhomogeneous RBC distributions in the presence of aberrant aggregation, (3) the wall shear stress (endothelial) environment.

In this study, RBC aggregation in the microcirculation was investigated through cell-level simulations, with a focus on its effects on cell distribution, cell-free layer dynamics, and

cell-wall interactions. Various influencing factors were considered, including cellular physical properties, flow conditions, vessel geometry, and blood disorders. Results indicate that RBC aggregation increases the average thickness of the cell-free layer due to mutual cell attraction, but also introduces substantial fluctuations in the cell-free layer, with more instances of erythrocytes clustering very close to the vessel wall. These cell clusters induce significant wall shear stress fluctuations, potentially damaging the glycocalyx layer. In cases involving blood disorders, where a small fraction of cells exhibit increased rigidity, erythrocyte aggregation enhances the segregation of various cellular components, while the margination of stiff cells and RBC aggregation together intensify wall stress fluctuations. Additionally, in curved vessels, larger cell-free void areas were observed in aggregation, suggesting that vessel geometry promotes cell clustering and contributes to spatial heterogeneity in the cell-free layer.

3.2 Formulation

We simulate a suspension of RBCs, modeled as deformable fluid-filled elastic capsules, flowing through rigid straight and curved cylindrical tubes. For blood disorders such as sickle cell disease, RBC suspensions are modeled as binary mixtures of normal and sickle RBCs. In these binary suspensions, the number fraction consists of 0.9 normal RBCs and 0.1 aberrant sickle RBCs. A suspension of normal RBCs without intercellular aggregation is considered a control. For most simulations here, the overall volume fraction (hematocrit), ϕ , of blood cells is around 20%, consistent with the hematocrit in the microcirculation [168, 169]. The suspending fluid, blood plasma, is considered incompressible and Newtonian with a viscosity of about $\eta = 1.10 - 1.35\text{mPas}$. In this study, we assume for computational reasons a viscosity ratio of 1 between the intercellular matrix and plasma. While typical RBCs may exhibit viscosity ratios of up to 15 [180], aberrant RBCs associated with blood disorders can display even higher values. Nevertheless, previous research has shown that the dynamics of both individual cells [222] and cell suspensions [223] remain qualitatively consistent across

a wide range of viscosity ratios. The discoid radius a for human RBC is about $4\mu\text{m}$. The RBC membrane in-plane shear elasticity modulus $G \sim 2.5 - 6\mu\text{N/m}$. The deformability of a capsule in the pressure-driven flow is measured by the dimensionless capillary number $Ca = \eta\dot{\gamma}_w a/G$. Ca is set to be 0.1 for normal RBCs, which corresponds to $\dot{\gamma}_w \sim 100\text{ s}^{-1}$. Cell membranes are modeled as isotropic, hyperelastic surfaces characterized by an interfacial shear modulus G , incorporating properties of shear elasticity, area dilatation, volume conservation, and bending resistance. The total strain energy of the RBC membrane S is expressed as:

$$E = \frac{K_B}{2} \int_S (2\kappa_H + c_0)^2 dS + \overline{K_B} \int_S \kappa_G dS + \int_S W dS$$

where K_B and $\overline{K_B}$ are the bending moduli, and W represents the shear strain energy density. Here, κ_H and κ_G denote the mean and Gaussian curvatures of the surface, respectively, while $c_0 = -2H_0$ represents the spontaneous curvature, with H_0 being the mean curvature of the spontaneous shape. The first two terms correspond to the Canham-Helfrich bending energy [224, 225], which accounts for the bending resistance of the membrane. The third term represents the shear strain energy stored in the RBC membrane, computed using the Skalak model [226]. More details of the membrane mechanics model, along with validation against experimental observations, are provided in [202, 227].

In this study, a normal RBC is modeled as a flexible capsule, with a spontaneous biconcave discoidal shape to present shear elasticity and an oblate spheroidal shape for bending elasticity [227, 228], with a characteristic radius of $a = 4\mu\text{m}$. In SCD, abnormal sickle hemoglobin polymerizes within RBCs upon deoxygenation, forming elongated fibers that disrupt the cellular architecture [127]. This pathological alteration increases membrane stiffness and leads to cellular dehydration, which in turn reduces cell volume. As a result, sickle RBCs are considerably less deformable than normal cells, with some subpopulations irreversibly adopting a sickle-like shape. In this study, we model a sickle cell as a stiff capsule with a curved prolate spheroidal rest shape. The volume of this curved prolate capsule is approximately 20% of that of the normal RBC model. Since aberrant RBCs in blood disor-

ders are generally much smaller and stiffer than normal RBCs, the interfacial shear modulus G of the aberrant RBCs is assumed to be five times that of normal RBCs, which leads to Ca for aberrant RBCs being at most 0.2 times that of Ca for normal RBCs. All results are for simulations that have been run to a statistically stationary state.

Fluid-structure interaction is addressed using the immersed boundary method (IBM). Specifically, our model incorporates two types of immersed boundaries: deformable moving cellular membranes and rigid nonmoving vascular walls. The capsule membrane is discretized into N_Δ piecewise flat triangular elements; $N_{\Delta p} = 1280$ for normal RBCs, while $N_{\Delta t} = 682$ for sickle RBC. Differing values of N_Δ are selected to ensure comparable sizes of triangular elements on both capsules. We employ the “continuous forcing” IBM and “direct forcing” IBM methods for the RBC membranes and vessel wall, respectively. The numerical methodology adheres to the approach delineated in previous works [178, 179]. Further details are found in the supplementary information of [202].

In this paper, the focus is on understanding the impact of RBC aggregation on hemodynamics in the microcirculation, rather than investigating the detailed mechanisms of RBC aggregation, thus a simple Morse potential is employed to model the interaction forces between RBCs[140]. In the numerical implementation, intercellular forces are applied between nodes on the triangular mesh representing the RBC membrane surface, so we define below the energy and force per unit area. The interaction energy per unit area for nodes separated by a distance r , denoted by $\varphi(r)$, is given by the expression:

$$\varphi(r) = D_e \left[e^{2\beta(r_0-r)} - 2e^{\beta(r_0-r)} \right]. \quad (3.1)$$

The corresponding force per unit area, $f(r)$, which describes the interaction forces, is obtained by taking the derivative of the potential:

$$f(r) = -\frac{\partial \varphi(r)}{\partial r} = 2D_e\beta \left[e^{2\beta(r_0-r)} - e^{\beta(r_0-r)} \right]. \quad (3.2)$$

Here, r_0 and D_e represent the zero force distance and surface energy scale per unit area, respectively, while β is the inverse interaction length. In our simulation, we set $r_0 = 0.49\mu m$ and $\beta = 3.84\mu m^{-1}$ [217]. To quantify the strength of aggregation relative to viscous stresses due to flow, we introduce the dimensionless number $K_{agg} = D_e\beta/\eta\dot{\gamma}_w$. Roughly speaking, at $K_{agg} = 1$, the forces pulling cells together are balanced by the viscous stress working to separate them. In the context of cell-cell interaction, to improve the computational efficiency, a cut-off length of $4\mu m$ is chosen, beyond which the attractive force rapidly diminishes. In the implementation, following finite element discretization of the RBC membrane, a sphere with a diameter equal to the cut-off length is utilized to identify the cell surface within the domain of influence around the node on the cell surface.

In our simulation, we maintain a particle Reynolds number, denoted as $Re_p = \rho\dot{\gamma}_w a^2/\eta$, at a fixed value of 0.1. Although this value of Reynolds number Re_p is high for microcirculation, it represents a compromise between physical accuracy and efficiency. To ensure the robustness of our findings, we performed verifications using $Re_p = 0.05$ and observed no changes in our conclusions. The fluid is assumed to be incompressible and Newtonian, thereby subjecting the flow to the governing equations of Navier-Stokes and continuity. No-slip boundary conditions are imposed on the tube walls, while periodic boundary conditions are applied in the flow direction. The cell suspension is subjected to unidirectional pressure-driven flow, with the velocity field in the absence of RBCs described by Poiseuille flow within a straight cylindrical tube. In this study, flow is driven by a constant pressure gradient, which is equivalent to fixing the mean wall shear rate at $\dot{\gamma}_w = 2U_0/R$, where U_0 is the undisturbed centerline velocity. For the curved cylindrical channel, pressure gradients are defined based on an equivalent straight cylindrical channel with the same centerline length and radius. Employing a projection method facilitates the temporal advancement of the velocity field. The cylindrical vessel is positioned within a cuboidal computational domain of $15a \times 10a \times 10a$, utilizing an Eulerian grid with dimensions $150 \times 100 \times 100$. For the curved vessel, a larger cuboidal computational domain of $26a \times 24a \times 10a$ is used, with an

Eulerian grid of dimensions $260 \times 240 \times 100$.

3.3 Results and discussion

3.3.1 Cylindrical tube

We begin the assessment of aggregation on cell dynamics within a suspension with simulations of RBC suspensions flowing through cylindrical tubes, as presented in Fig. 3.1. Fig. 3.1(A) presents simulation snapshots of RBC suspensions with and without aggregation, at a 20% volume fraction, flowing through a cylindrical channel. In the suspension without RBC aggregation ($K_{\text{agg}} = 0$), RBCs are more evenly distributed within the channel, concentrating primarily in the tube center and forming a cell-free layer along the vessel wall. In contrast, when RBC aggregation occurs ($K_{\text{agg}} = 1$), the cells aggregate into stacks, creating rouleaux-like structures and resulting in large void regions due to the attraction among cells. In the microcirculation, normal RBCs tend to migrate away from the vessel wall, forming a cell-free layer that is governed by the interaction of two cross-stream fluxes: wall-induced migration and hydrodynamic collisions[159]. The thickness of the cell-free layer varies over time and across different spatial locations near the vessel surface. To quantitatively evaluate the thickness and its variations, we define it as the distance from the center-of-mass position of the cell closest to the wall to the vessel surface, as indicated by the dashed line in Fig. 3.1 (A). The results from Fig. 3.1 (A) qualitatively show that in the absence of RBC aggregation, the thickness of the cell-free layer remains relatively uniform. However, with aggregation, the cell-free layer becomes highly heterogeneous, exhibiting substantial fluctuations in its thickness.

To provide further insight into the CFL, a point is randomly placed on the channel surface, and the thickness of the cell-free layer nearest to this point is measured over time as blood suspension flows. Fig. 3.1 (B) presents the temporal variation of the cell-free layer thickness near a randomly selected fixed point on the cylindrical vessel surface. It was observed

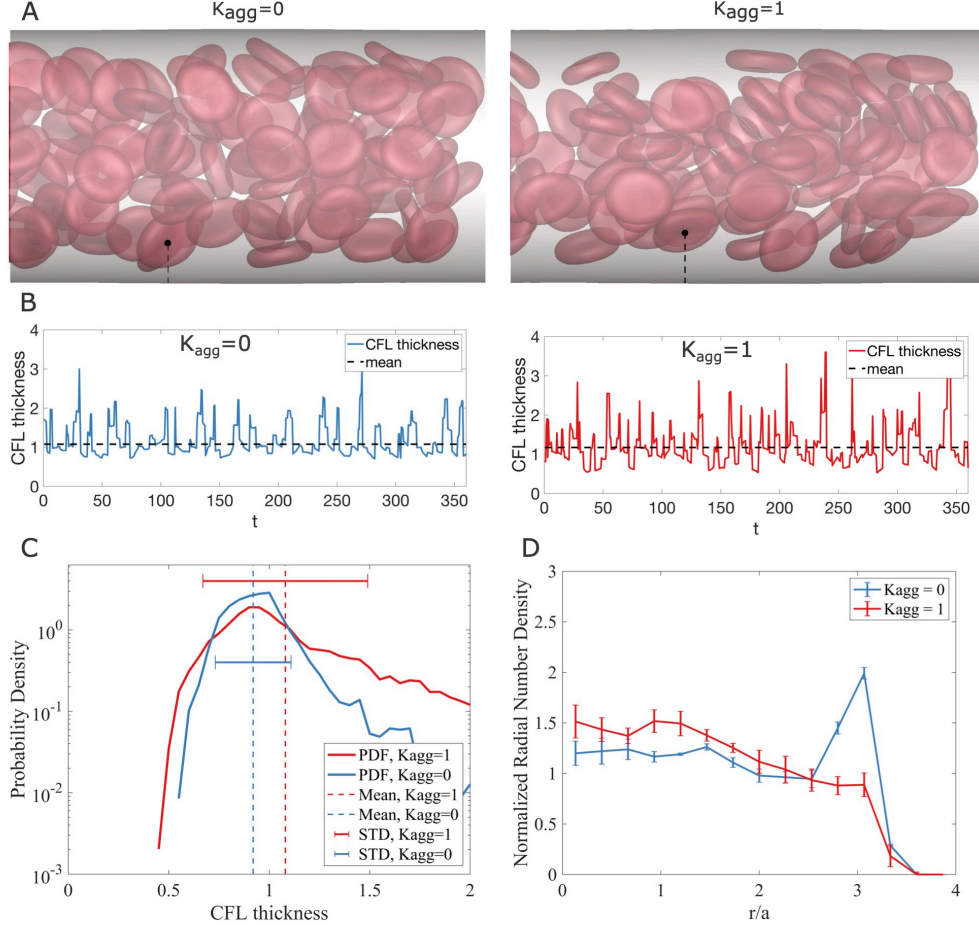


Figure 3.1: (A) Simulation snapshots of a suspension of RBCs flowing with (left) $K_{agg} = 0$ and (right) $K_{agg} = 1$ through a cylindrical tube with a radius of $16\mu m$. The direction of flow is from left to right. Hematocrit $\phi = 20\%$. Dots and dashed lines represent the position of the cell center-of-mass and the thickness measurements of the nearby cell-free layer, respectively. (B) Temporal evolution of cell-free layer thickness near a fixed point on the cylindrical surface in the cell suspension with (blue) $K_{agg} = 0$ and (red) $K_{agg} = 1$. (C) Probability density profile of CFL thickness near the cylindrical surface. (D) Normalized radial number density profile for blood cells in a cylindrical tube. The number density is counted based on the cell center-of-mass position. The profile is normalized such that if the cells are evenly distributed in the tube, then the normalized cell number density is one everywhere.

that in the presence of aggregation, the average thickness of the cell-free layer increased, accompanied by a notable amplification in the fluctuation of its thickness. Thus, we observed spatiotemporal non-uniformity in the cell-free layer due to the emergence of cell aggregates, leading to instances where certain cells closely approach the vessel wall. It is important to note that although the CFL thickness is calculated based on the cell center-of-mass position illustrated in Fig. 3.1(A), accounting for the cell radius, the actual proximity of the cell to the wall can be significantly close. In the presence of cell aggregation, the average thickness of the cell-free layer increases, as expected, due to the tendency of cells to attract one another rather than stay close to the vessel wall. However, the variance is also larger, reflecting the formation of cell aggregates. Experiments by Druzak et al. [118] demonstrated that both the number and size of RBC aggregates increase with rising fibrinogen concentration. In their microfluidic channels, fibrinogen-induced erythrocyte aggregation resulted in glycocalyx reduction and damage to the vascular endothelium.

However, the mechanisms underlying the resulting endothelial damage remain unclear. To investigate this in a computational model, we conducted a statistical analysis of the variations in cell-free layer thickness. Fig. 3.1(C) displays the probability density curve of the cell-free layer thickness. Notably, the probability density profile for the cell-free layer thickness exhibited a broader distribution in the scenario of cell aggregation. This implies that particularly for very thin cell-free layers, the probability of occurrence is higher when aggregation is present compared to when it is absent, despite the fact that cell aggregation results in an overall increase in the average thickness of the cell-free layer. To describe the statistical distribution of a suspension of cells during flow, the radial distribution of cells within the tube is depicted in Fig. 3.1(D). It was observed that in scenarios involving cell aggregation, the peak of cell density adjacent to the cell-free layer decreased, whereas the concentration of cells around the central region of the tube increased. Moreover, in instances of aggregation, there was an augmented deviation in cell distribution, affirming the presence of distribution heterogeneity induced by aggregation.

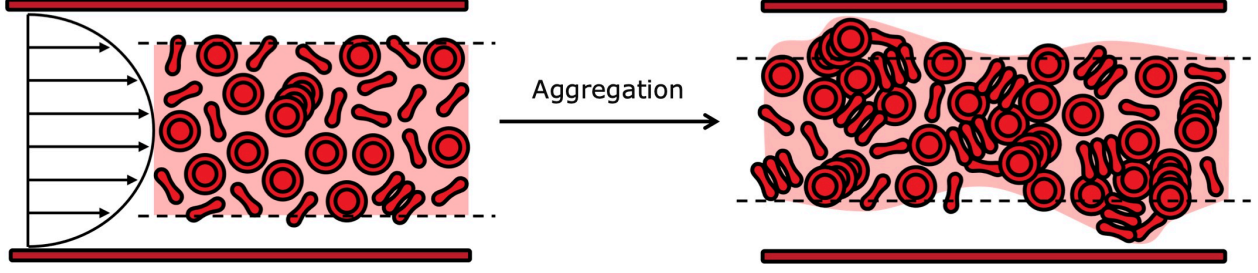


Figure 3.2: Schematic diagram showing the process of RBC aggregation in the blood flow. Nearby red blood cells aggregate into a clump or rouleaux shape as a result of mutual attraction ; As a result, in the presence of cell aggregation, the average thickness of the CFL increased, while CFL fluctuations became more pronounced, and cell aggregates formed very close to the vessel wall.

Based on our simulation findings, we propose a hypothesis suggesting that aggregated RBCs form rouleaux or clusters during flow, as shown in Fig. 3.2. These clusters approach the blood vessel wall closely, potentially even surpassing the thickness of the cell-free layer in the absence of aggregation. This direct physical contact is posited to induce fluctuations in wall stress, ultimately contributing to the reduction of the glycocalyx and subsequent vascular damage. To study the hydrodynamic impact of cell aggregation on the vascular surface, we now characterize the wall shear stress environment experienced without and with aggregation. Simulation snapshots illustrating wall shear stress are presented in Fig. 3.3 (A,B). These reveal that local stress fluctuations are more pronounced in RBC suspensions with aggregation than without. Transparent views in Fig. 3.3 (A) illustrates that RBC aggregates closer to the wall, leading to heightened local stress fluctuations. Conversely, without aggregation, the CFL maintains greater uniformity, thus mitigating stress fluctuations attributed to RBCs being in close proximity to the wall. For better elucidation, Fig. 3.3 (C) demonstrates the temporal variation of wall stress at a fixed point on the vascular surface. At certain time intervals, the aggregated RBC suspension induces large upward fluctuations in wall stresses due to the proximity of a cell or aggregate. This phenomenon is further illustrated by the probability density profile depicted in Fig. 3.3 (D), where a substantial disparity in the high-stress tail of the distribution highlights that the likelihood of encountering high wall shear stress in aggregating RBC suspensions is orders of magnitude greater.

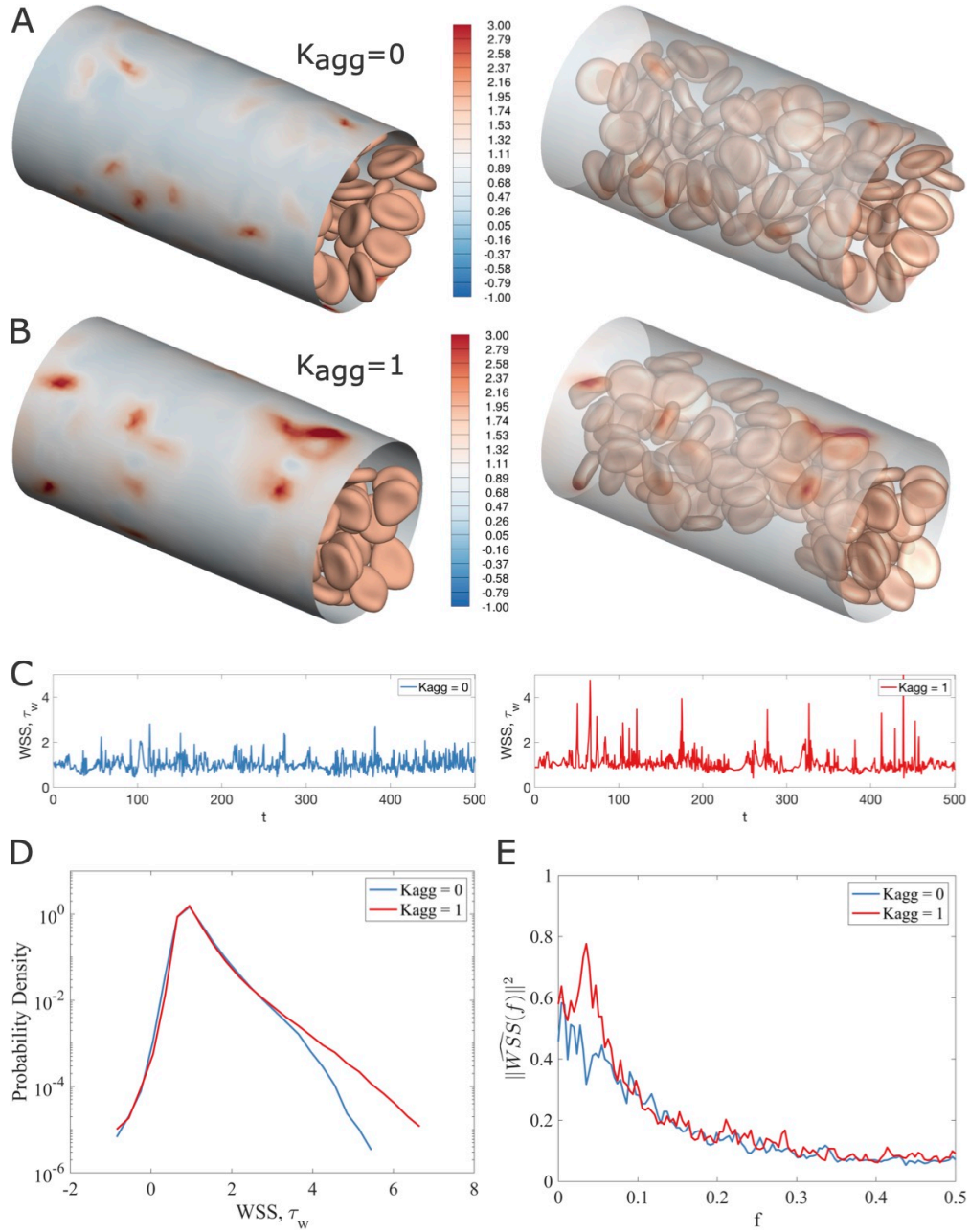


Figure 3.3: Simulation snapshots of (left) wall shear stress on the cylindrical surface and (right) corresponding transparent views in RBC suspension with (A) $K_{agg} = 0$ and (B) $K_{agg} = 1$. (C) Temporal evolution of wall shear stress at a fixed point on the cylindrical surface in RBC suspension. (D) Probability density profile of wall shear stress on the cylindrical surface. Note that the wall shear stress is dimensionless using $\dot{\gamma}_w \sim 100 \text{ s}^{-1}$. (E) Power spectral density of wall shear stress extracted with the mean, $\tau_w - \bar{\tau}_w$. This spectral analysis is performed using a Hamming window.

Fig. 3.3(E) compares the power spectral density of the wall shear stress (WSS) fluctuations at a point on the wall (averaged over many points) without and with aggregation. In the present analysis, time is scaled with inverse shear rate so a frequency $f = 1$ is a dimensional frequency of $\dot{\gamma}$. The results indicate that for $f \gtrsim 0.3$, the spectrum is fairly flat, corresponding to fluctuations due to the overall randomness of the flow in the presence of RBCs and their interactions.

Some scaling arguments lead to further insight into the behavior at lower frequencies. We can estimate the streamwise velocity v of cells interacting with the wall to scale as $v \sim \dot{\gamma}a$ – this is the fluid velocity of one particle radius a from the wall. The streamwise flux j_w of cells near the wall satisfies $j_w \sim nv$, where n is the cell number density. This obeys $n \sim \phi a^{-3}$, where ϕ is volume fraction (hematocrit). With this result, the streamwise flux $j_w \sim \phi a^2 \dot{\gamma}$. With these results, the frequency f_w of cells passing within one radius of a particular point on the wall can be estimated as $f_w \sim j_w a^2$, leading to the final estimate $f_w \sim \phi \dot{\gamma}$. We interpret the increase in the PSD for $f \lesssim 0.3$ as the effect of individual cells moving by the sampling point. Some fraction of these will pass close enough to lead to a spike in WSS. For a smooth CFL, ($K_{agg} = 0$) there is no further structure. In contrast, for $K_{agg} = 1$, we see a fairly distinct peak centered near $f = 0.04$; this probably reflects the average wavelength of the fluctuations in the CFL corresponding to aggregated regions of RBCs passing very close to the wall. This point is corroborated by examination of the $K_{agg} = 1$ plot on Fig. 3.3(C); here there are roughly 18 events with $WSS > 2$ over 500 time units, corresponding to a frequency of $18/500 = 0.036$, very close to the peak in the PSD.

3.3.2 Combination of Aggregation and Marginating Sick Cells

Recent literature [202] reports that abnormal cells in blood disorders frequently marginate near the vessel wall, generating high wall stresses and potentially causing vessel damage through direct physical contact. However, the influence of cellular aggregation on the segregation process remains insufficiently explored and merits further investigation. To address

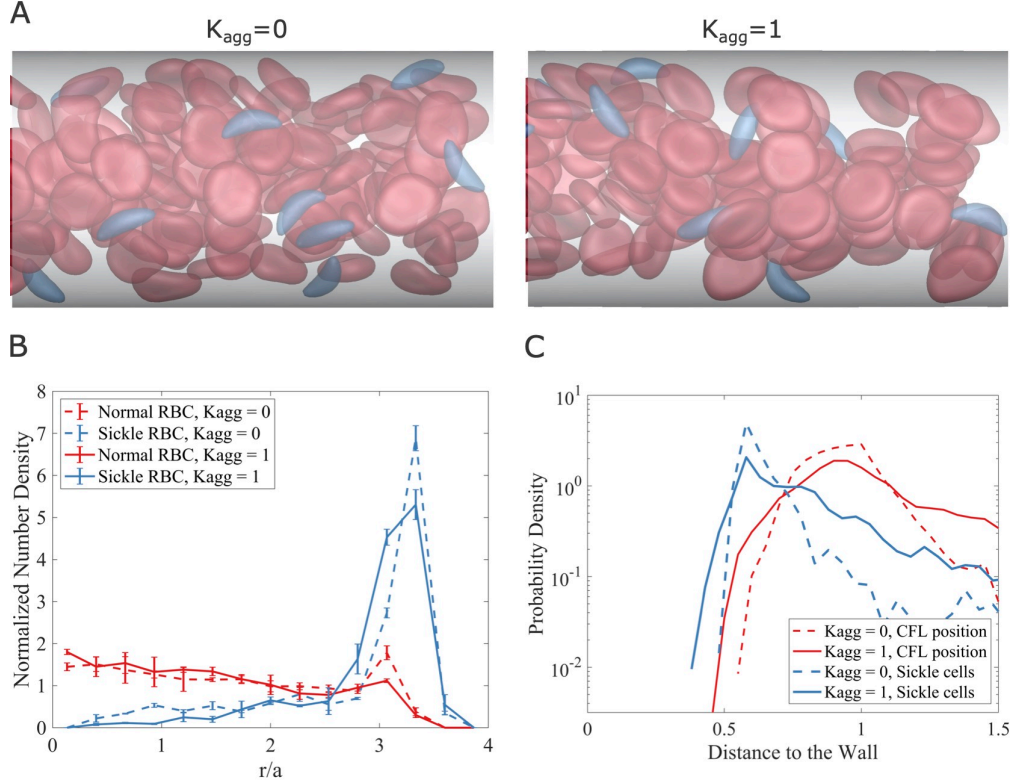


Figure 3.4: (A) Simulation snapshots of a binary suspension of normal RBCs with sickle cells flowing with (top) $K_{agg} = 0$ and (bottom) $K_{agg} = 1$ through a cylindrical tube. Hematocrit is set to 20%. (B) The radial cell number density distribution of (red) normal RBCs and (blue) sickle cells within the suspension with (solid line) aggregation and without (dash line) aggregation. (C) Probability density profiling for the distance of (blue) sickle cells and (red) CFL to the walls in the suspension (solid line) with aggregation and (dash line) without aggregation.

this, we developed a simplified computational model to study blood flow in the context of SCD, building on our previous studies [202]. RBC suspensions are modeled as binary mixtures of normal RBCs and aberrant RBCs consisting of 90% normal RBCs and 10% irreversibly sickled cells. The introduction of irreversibly sickled cells adds two additional types of cell-cell aggregation interactions: sickle cell-to-sickle cell and sickle cell-to-normal cell. It is known that sickle cells become sticky and can block the vessels. Nevertheless, for simplicity and to isolate the interplay of margination and aggregation, in this study we apply the same cell-cell aggregation model between all cells.

A comparative analysis was conducted for cases with SCD RBCs as illustrated in Fig. 3.4. The simulation snapshots in Fig. 3.4(A) illustrate the flow of the SCD RBC suspension

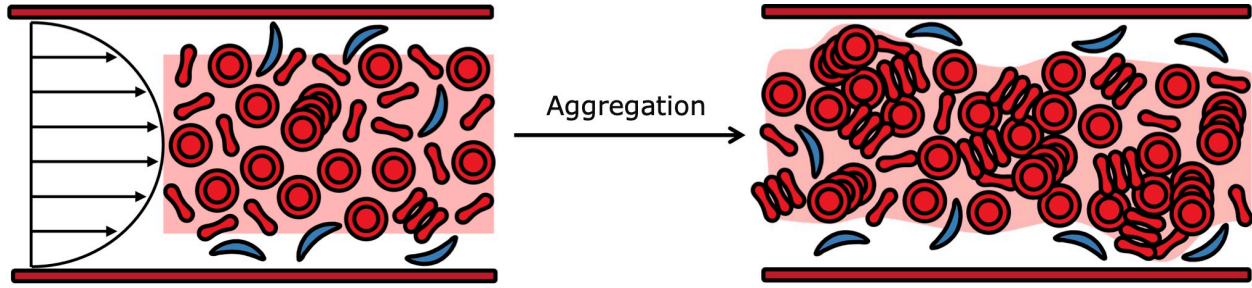


Figure 3.5: Schematic showing the cell segregation in the aggregation. The perturbation of cell-free layer thickness in the case of RBC aggregation can increase the probability of sickle cell close to the walls.

through a cylindrical channel, comparing scenarios with and without cell aggregation. In the absence of cell aggregation, a uniform cell-free layer is formed by the normal RBC migration, while the stiff sickle cells tend to marginate closer to the vessel wall. With aggregation, however, the mutual attraction between cells results in a more compact distribution of RBCs, while the phenomenon of cell segregation remains remarkable. For quantitative analysis, Fig. 3.4 (B) presents the radial distributions of normal and sickle cells. In the absence of aggregation, normal cells exhibit a relatively higher concentration peak near the vessel wall. In contrast, with aggregation, normal cells are more concentrated within the center of the channel. When aggregation is absent, sickle cells are primarily positioned near the vessel wall. However, in the presence of aggregation, the mutual attraction among normal RBCs leads to clusters occupying the channel center, thereby boosting the margination and resulting in a higher proportion of sickle cells within the cell-free layer. In addition, a statistical analysis of cell distribution near the vessel wall is presented in Fig. 3.4(C), showing the probability distributions for the locations of the cell-free layer and sickle cells. The majority of sickle cells are positioned outside the cell-free layer. Although cell aggregation induces fluctuations within the cell-free layer, resulting in a broader probability distribution, it also increases the likelihood of sickle cells approaching very close to the vessel wall. This is due to aggregation-induced fluctuations in the CFL thickness, which increase the perturbation in the spatial distribution of sickle cells within CFL, thereby elevating the likelihood of their proximity to the vessel wall, as illustrated in the schematic in Fig. 3.5.

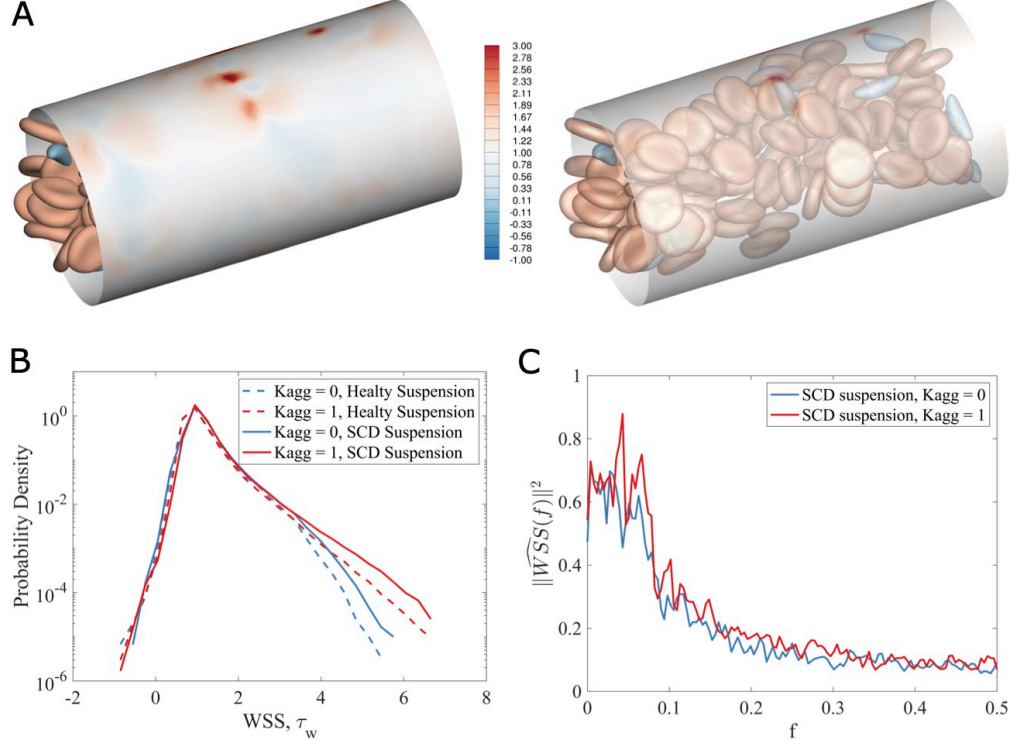


Figure 3.6: (A) Simulation snapshots of (top) wall shear stress on the cylindrical surface and (bottom) corresponding transparent views in SCD RBC suspension with $K_{agg} = 1$. (B) Probability density profile of wall shear stress τ_w on the vascular surface. (C) Power spectral density of wall shear stress extracted with the mean, $\tau_w - \bar{\tau}_w$, in SCD suspension.

To characterize the hydrodynamic effects of SCD RBC aggregation on the vascular surface, Fig. 3.6(A) presents the spatial distribution of wall shear stress. The transparent view on the right reveals that cell aggregation causes the cell-free layer to become highly inhomogeneous. As cells aggregate into clusters, nearby stiff margined sickle cells approach closely the vessel wall, resulting in large local fluctuations in wall shear stress. Fig. 3.6(B) displays the probability distribution of wall shear stress for four different suspensions – SCD and healthy RBCs, both with and without aggregation. These results indicate that the combination of cell aggregation and the presence of sickle cells leads to enhanced fluctuations in wall shear stress and an increased probability of high wall stress events. Fig. 3.6(C) compares the PSD of wall shear stress fluctuations for SCD RBC suspensions with and without aggregation. Unlike in pure normal RBC suspensions, the case with $K_{agg} = 1$ exhibits two distinct peaks, centered at around $f = 0.04$ and $f = 0.07$. The peak at $f = 0.04$ observed in

the absence of sickle cells persists, with about 10% larger amplitude, while in the presence of sickle cells, a higher-frequency peak, at $f = 0.07$, is observed as well. This high-frequency peak may arise from the synergistic effect of cell aggregation generating perturbations within the CFL, implying that cell aggregation increases the interaction of sickled cells with the vessel wall.

3.3.3 Effect of Membrane Deformability and Hematocrit in Aggregation

In the prior sections, the capillary number for the normal RBCs was set to 0.1, corresponding to a wall shear rate of approximately 100 s^{-1} . Here, the effect of RBC aggregation at an increased capillary number of 1 is analyzed, as shown in Fig. 3.7. We continue to consider the cases $K_{agg} = 0$ and $K_{agg} = 1$. Recalling the definitions $Ca = \eta\dot{\gamma}a/G$ and $K_{agg} = D_e\beta/\eta\dot{\gamma}_w$, increase Ca by increasing shear rate, to keep K_{agg} constant we need to also increase D_e . I.e. we are increasing shear rate, while keeping the balance between aggregation and viscous stress constant.

Fig. 3.7(A) shows that cells at a higher capillary number ($Ca = 1$) exhibit greater deformations than at $Ca = 0.1$ (as expected), and an apparently more homogeneous cell-free layer compared to those in previous simulations. The latter observation is further supported in Fig. 3.7(B), the probability distribution of cell-free layer thickness, demonstrating that at $Ca = 1$, the cell-free layer is generally thicker, and the difference in statistical distribution between the cell-free layer with and without RBC aggregation is relatively small. We attributed this small difference to the stronger wall-induced migration at the higher Ca . Nevertheless, an increased probability of a thinner cell-free layer persists when $K_{agg} = 1$. Fig. 3.7(C) compares the radial distribution of cell number density for cases with and without cell aggregation. In the absence of aggregation, a distinct peak appears near the wall, while in the presence of aggregation, this peak decreases as cells tend to concentrate toward the channel center. Fig. 3.7(D) displays the probability distribution of wall shear stress.

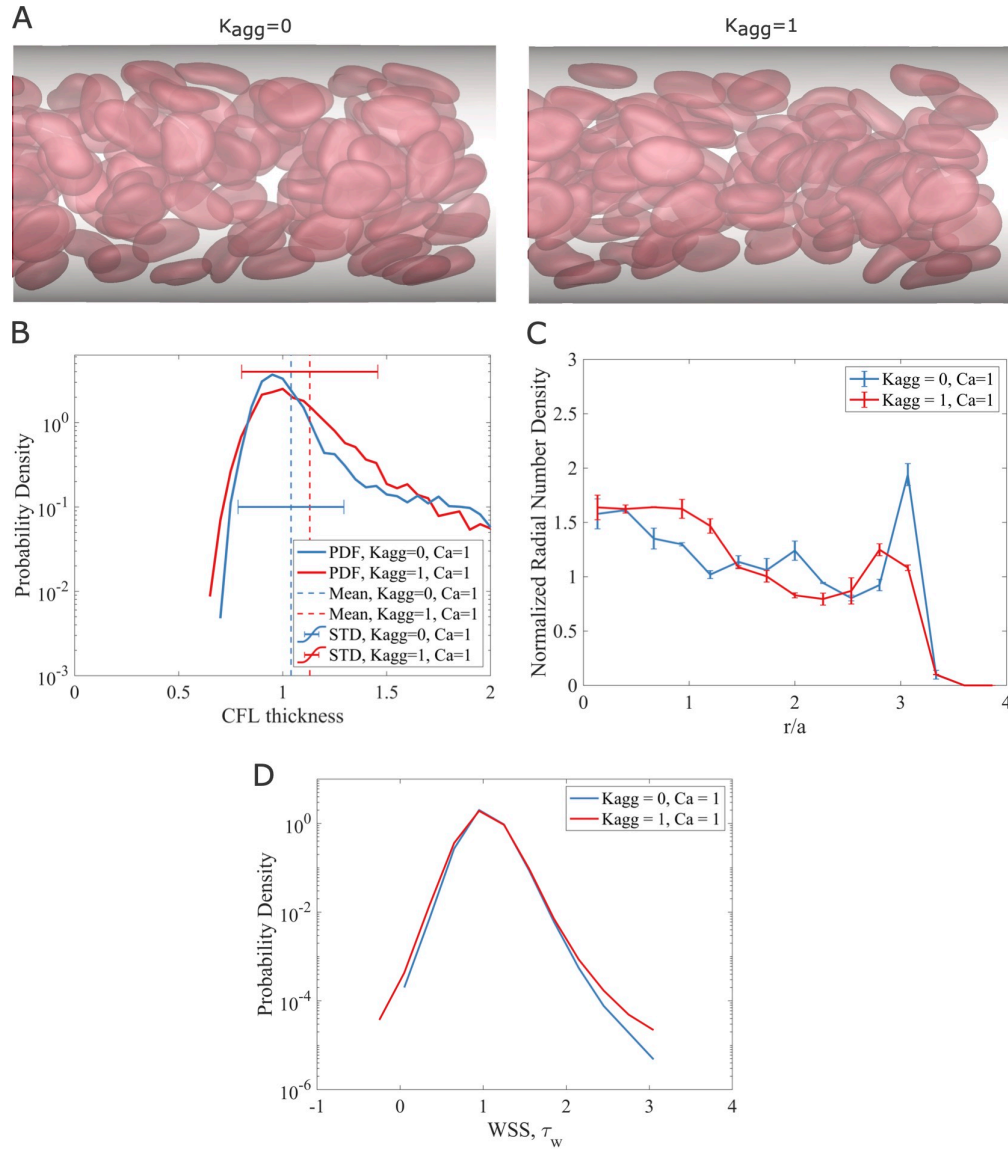


Figure 3.7: (A) Simulation snapshots of a suspension of RBCs with $Ca = 1.0$ flowing at (left) $K_{agg} = 0$ and (right) $K_{agg} = 1$ through a cylindrical tube with a radius of $16\mu m$. Hematocrit is set to 20%. (B) Probability density profile of CFL thickness on the cylindrical surface. (C) Normalized radial number density profile for RBCs in a cylindrical tube. (D) Probability density profile for the wall shear stress τ_w on the vascular surface.

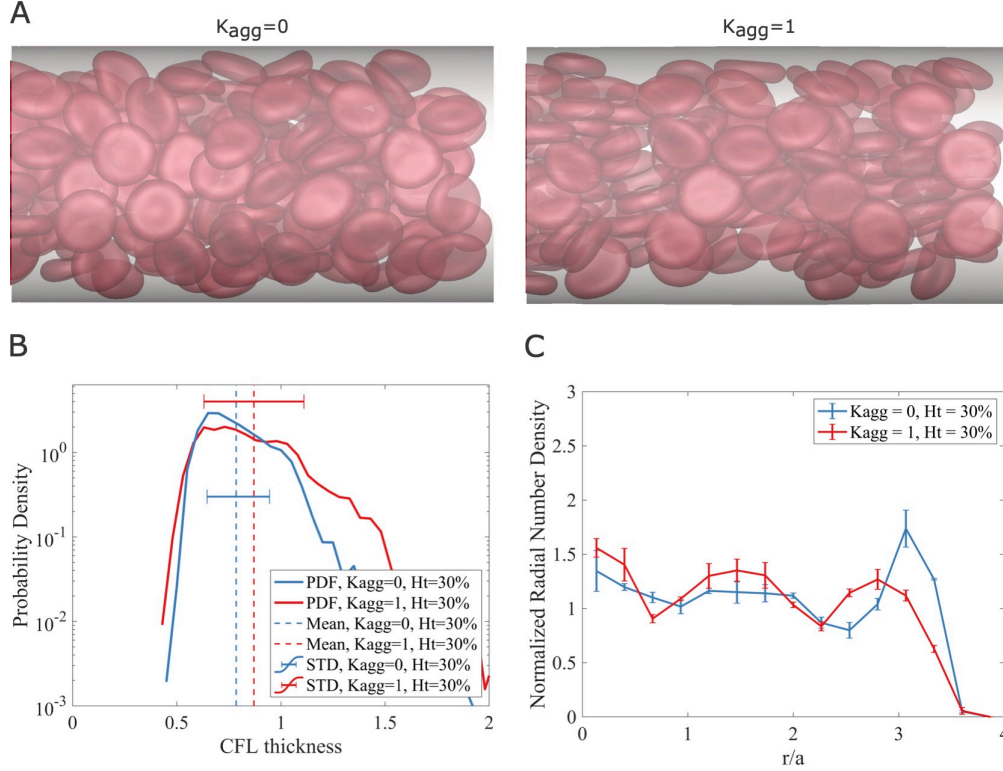


Figure 3.8: (A) Simulation snapshots of a suspension of RBCs with $Ca = 0.1$ and hematocrit $Ht = 30\%$ flowing at (top) $K_{agg} = 0$ and (bottom) $K_{agg} = 1$ through a cylindrical tube. (B) Probability density profile of CFL thickness on the cylindrical surface. (C) Normalized radial number density profile for blood cells in a cylindrical tube.

Although aggregation slightly increases the probability of higher wall shear stress, the effect remains limited; the cell-free layer is much thicker at $Ca = 1$ than at $Ca = 0.1$, and aggregation does not substantially influence the wall shear stress distribution. In summary, at high Ca , cell distribution, and wall shear stress statistics are less sensitive to the presence or absence of aggregation, compared with at low Ca .

We now consider the effect of volume fraction. Hematocrit is not a simple constant in the microcirculation, making it essential to understand the influence of RBC volume fraction on cell aggregation. To address this issue, we now present the distributions and dynamics of RBCs under aggregation conditions, as well as the associated wall stresses, at volume fraction $\phi = 30\%$. Simulation snapshots in Fig. 3.8(A) show a reduced cell-free layer thickness, as expected at higher ϕ [200]. In the presence of cell aggregation, we still observe

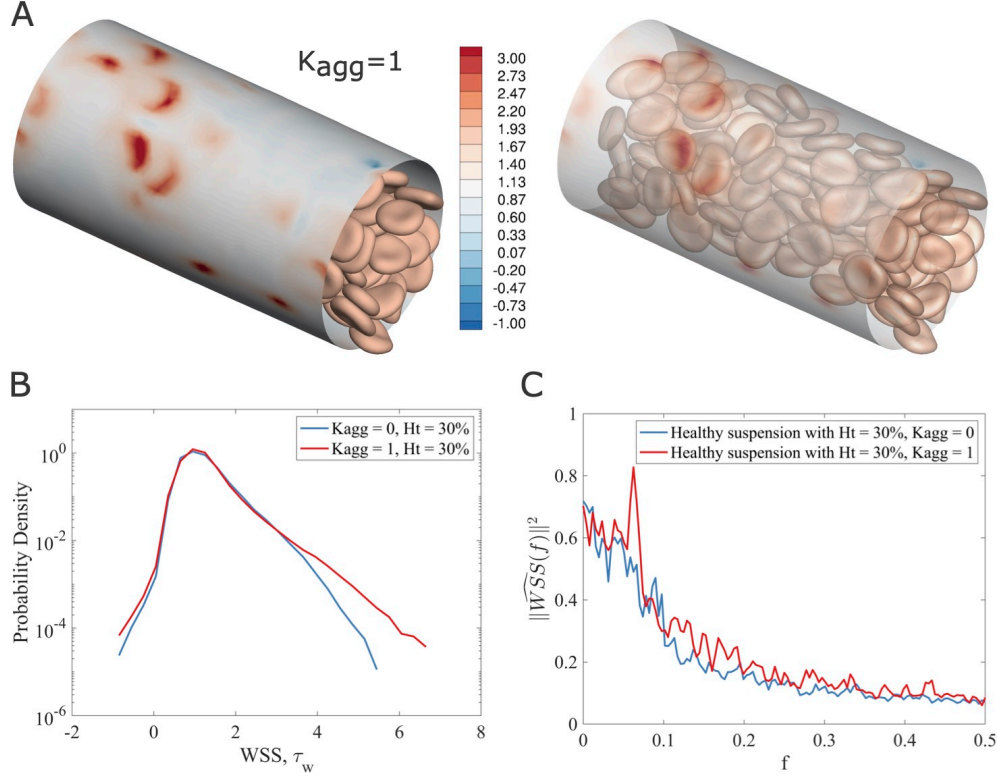


Figure 3.9: (A) Simulation snapshots of (top) wall shear stress on the cylindrical surface and (bottom) corresponding transparent views in RBC suspension with intercellular aggregation $K_{agg} = 1$, $Ca = 0.1$, and high hematocrit $Ht = 30\%$. (B) Probability density profile for wall shear stress on the vascular surface in the RBC suspensions with and without aggregation. (C) Power spectrum density of wall shear stress extracted with the mean $\tau_w - \bar{\tau}_w$ in RBC suspension at 30% hematocrit.

the formation of rouleaux as well as spatial fluctuations in the cell-free layer; however, unlike at lower hematocrit levels, very large voids of cells do not appear. To further quantify these observations, the probability density distribution of cell-free layer thickness at high hematocrit is presented in Fig. 3.8(B). Results indicate that at high hematocrit, the cell-free layer is, on average, thinner than at low hematocrits, as expected due to the increased volume occupied by RBCs. Analysis of the cell-free layer dynamics reveals that, even in the presence of aggregation, instances of a very thin cell-free layer still occur. However, the standard deviation of the cell-free layer thickness is reduced at high hematocrits in both the aggregation and non-aggregation cases.

The effect of high hematocrit on vascular wall stress is also examined, as shown in Fig. 3.9. In Fig. 3.9 (A), it is observed that at high hematocrit, the cell-free layer becomes thinner,

allowing cells to come into closer proximity with the wall, leading to localized stress fluctuations. Fig. 3.9 (B) compares cases with and without cell aggregation at high hematocrit, revealing that higher cell concentrations promote aggregation, thereby increasing the likelihood of large stress fluctuations. In Fig. 3.9 (C), the PSD of wall stress is compared. As at $\phi = 0.2$, under conditions of aggregation, the PSD displays a pronounced peak, now at around $f = 0.06$ rather than $f \sim 0.04$, again suggesting that clusters of RBCs contribute to stress fluctuations along the vascular wall. Notably, the 50% increase in the peak frequency with a 50% increase in ϕ is consistent with the physical scaling argument given above, which indicated that $f \sim \phi^1$.

3.3.4 Effect of Vascular Geometry: Geometric complexity facilitates RBC clustering

In the microcirculation, blood vessels are not simple cylindrical tubes; rather, they exhibit curvature and branching, forming complex vascular networks. Thus, the interaction between vascular geometry and RBC aggregation is studied in this work, as depicted in Fig. 3.10. Both aggregation and non-aggregation conditions are considered with Capillary number $Ca = 0.1$ and hematocrit 20%. The simulation snapshots in Fig. 3.10(A) reveal that, in the absence of aggregation, RBCs within the curved channel are more uniformly distributed. In contrast, when aggregation occurs, RBCs form rouleaux, aggregates, and even clusters, leading to a markedly heterogeneous spatial distribution with substantial void regions throughout the curved channel. Interestingly, in the case of $K_{agg} = 1$, the disruption of the "ribbon" structure formed by aggregated RBCs is observed, highlighting the influence of the curved geometry on RBC aggregation and promoting further cell clustering.

To further characterize the spatial distribution of RBCs within the curved channel, Figures 3.10(B) and 3.10(C) present the cell number density distribution in the center plane and cross-section of the curved channel, respectively. Unlike the straight cylindrical tube, the cell-free layer thickness varies across regions of the curved channel, with the outer side ($\theta < \pi/2$)

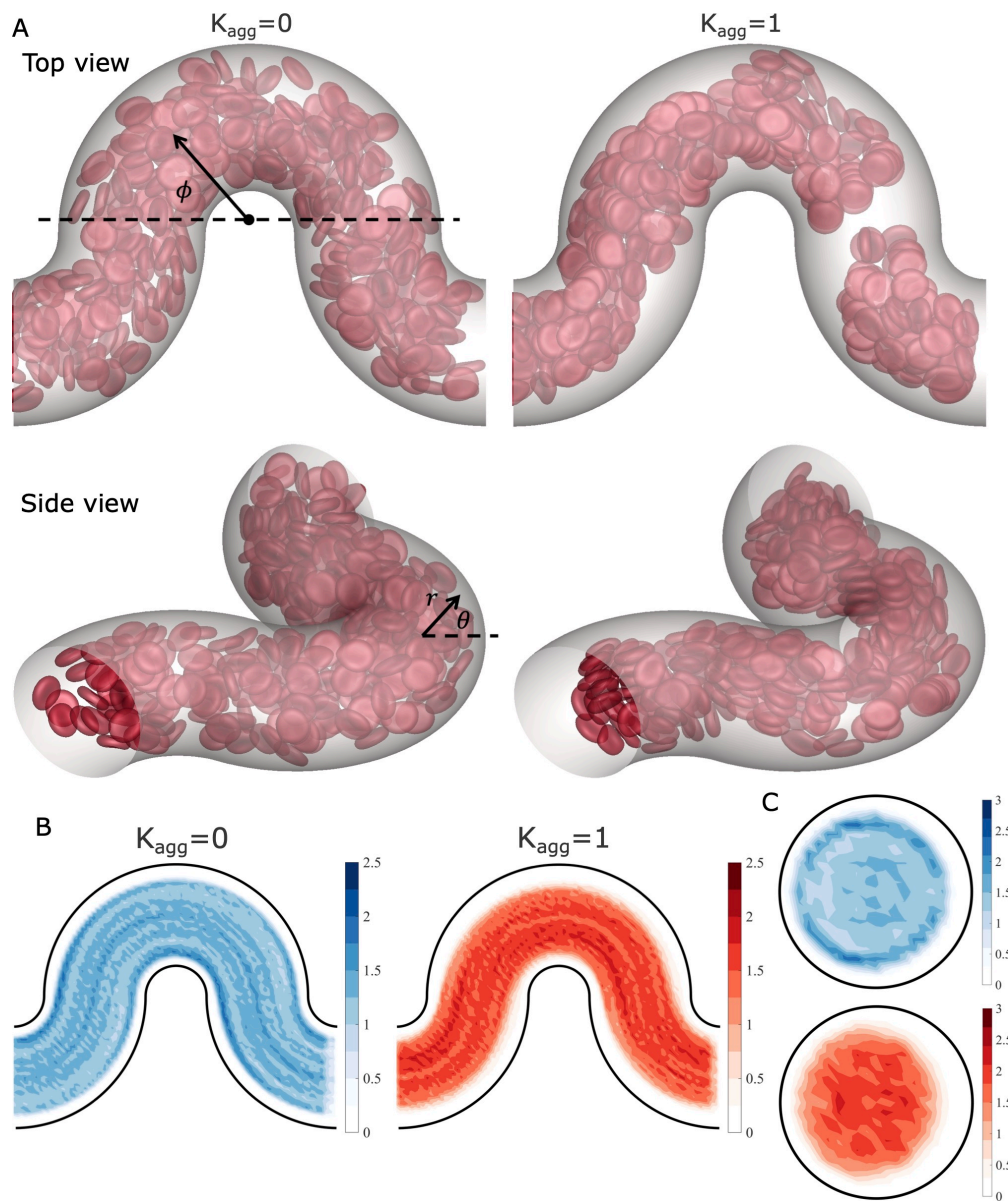


Figure 3.10: (A) Simulation snapshots of a suspension of RBCs flowing with (left) $K_{agg} = 0$ and (right) $K_{agg} = 1$ through a curved cylindrical tube. Hematocrit, the volume fraction of blood cells, is set to 20%. Capillary number Ca is set to 0.1. The flow direction is from left to right. (B) Center-plane cell number density distribution in a curved channel. (C) Cross-sectional ($0 < \phi < \pi$) cell number density distribution in a curved channel.

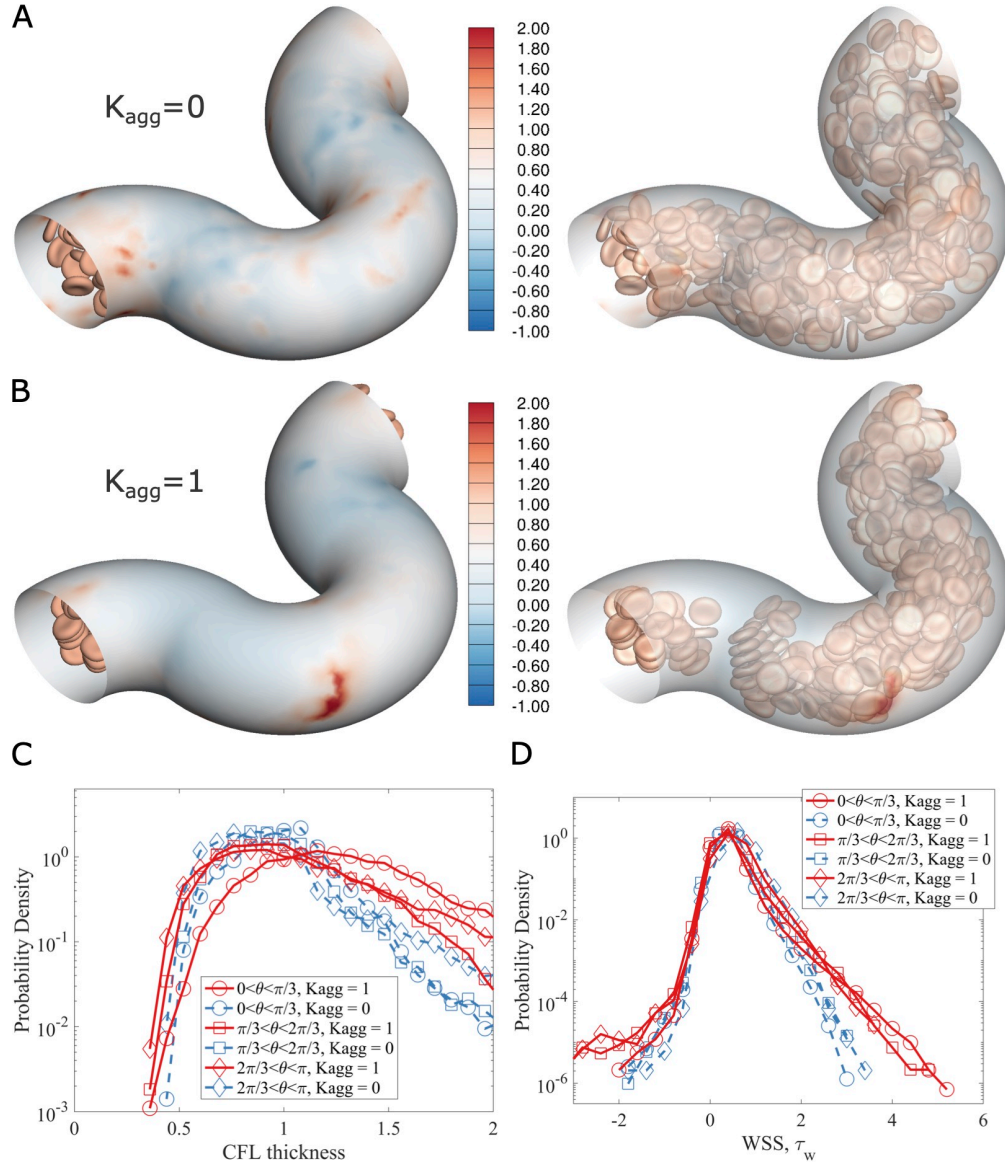


Figure 3.11: Simulation snapshots of (left) wall shear stress on the curved surface and (right) corresponding transparent views in RBC suspension with (A) $K_{agg} = 0$ and (B) $K_{agg} = 1$. (C) Probability density profile of CFL thickness on the curved surface at different θ . (D) Probability density profile of wall shear stress on the curved surface within different regions, $0 < \theta < \pi/3$ on the outside, $\pi/3 < \theta < 2\pi/3$ in the middle, and $2\pi/3 < \theta < \pi$ on the inside.

of the curve exhibiting a thicker cell-free layer compared to the inner side ($\pi/2 < \theta < \pi$). RBC aggregation increases the time-averaged thickness of the cell-free layer in the curved channel while also introducing greater spatial variability. These findings suggest that the geometric complexity of the vessel promotes both instantaneous and time-averaged heterogeneity in the cell-free layer, resulting in larger void regions devoid of cells and encouraging the formation of RBC clusters and clumping during aggregation.

To investigate the hydrodynamic effects of cells on curved vascular surfaces, shear stress distributions along the vascular wall were analyzed, as shown in Fig. 3.11(A, B). Notably, Figure 3.11(B) reveals that RBC aggregation induces large fluctuations in local wall shear stress, particularly along the outer curvature of the vessel, where RBC aggregates accumulate near the wall. Statistical analyses of the cell-free layer thickness and wall shear stress in the inner, middle, and outer regions of the curved channel, depicted in Fig. 3.11(C, D), further elucidate these effects. RBC aggregation broadens the probability distribution of the cell-free layer thickness across all regions in Fig. 3.11(C), signifying heightened perturbations and an increased likelihood of very thin cell-free layers. The instantaneous distribution of cell-free layer thickness is highly non-uniform, characterized by regions devoid of cells and areas where aggregates approach the wall. In addition, Fig. 3.11(D) demonstrates that RBC aggregation elevates the probability of high wall shear stress, particularly on the outer surface of the curved vessel wall, corroborating the localized fluctuations observed in Fig. 3.11(B). These results highlight the critical role of RBC aggregation in shaping the hydrodynamic environment of curved vascular.

The aggregation behavior of our model SCD RBC suspension in a curved channel was also investigated, as shown in Fig. 3.12(A). Similar phenomena were observed in the curved channel, where RBCs attracted each other, and aggregation led to fluctuations in cell-free layer thickness, boosted the segregation between normal RBCs and sickle cells, and increased the likelihood of these marginating sickle cells being in close proximity to the vessel wall. The distribution of wall shear stress on the curved channel surface in the SCD RBC suspension is

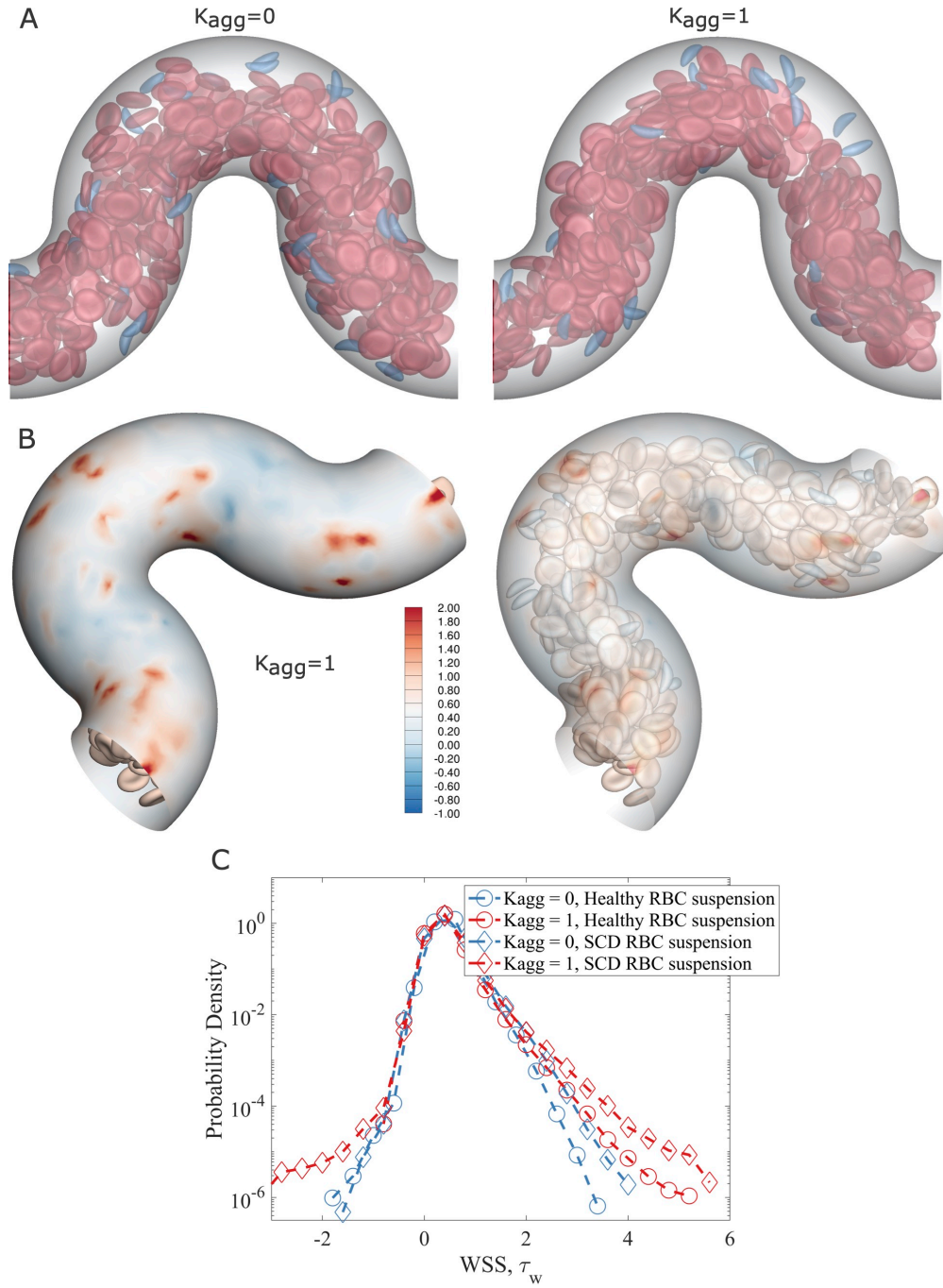


Figure 3.12: (A) Simulation snapshots of a suspension of SCD RBCs flowing with (left) $K_{agg} = 0$ and (right) $K_{agg} = 1$ through a curved channel. (B) Wall shear stress distribution on the curved vessel surface and its corresponding transparent view in the suspension of SCD RBCs with $K_{agg} = 1$. (C) Probability density profile of wall shear stress on the entire curved surface.

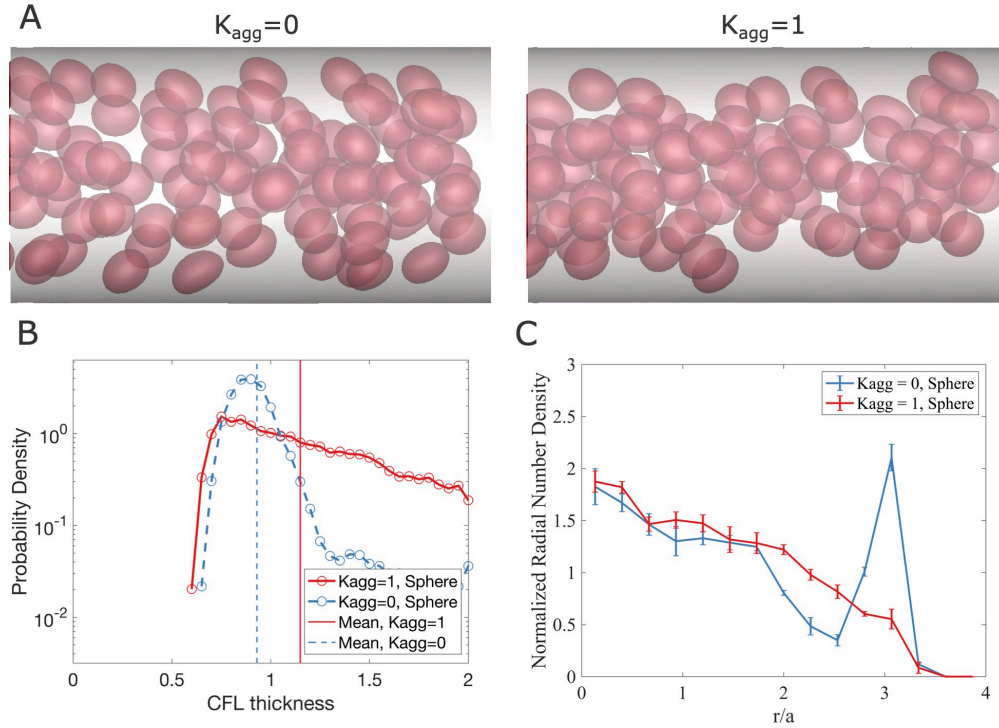


Figure 3.13: (A) Simulation snapshots of a suspension of spherical capsules flowing with (left) $K_{agg} = 0$ and (right) $K_{agg} = 1$ through a cylindrical tube. Hematocrit, the volume fraction of blood cells, is set to 20%. The volume of each spherical capsule is set equal to the volume of RBC with a biconcave discoid shape, so the radius of the spherical capsule is close to $2.8 \mu m$. (B) Probability density profile of CFL thickness on the cylindrical surface in the suspension of spherical capsules. (C) Normalized radial number density profile for spherical capsules in a cylindrical tube.

shown in Fig. 3.12(B). Observations from the transparent view revealed that the combination of RBC aggregation and stiff sickle cell margination resulted in large fluctuations in wall shear stress, contributing to vascular damage and inflammation. A statistical analysis of wall shear stress in the curved channel, presented in Fig. 3.12(C), indicates that the synergistic effects of RBC aggregation and sickle cell margination substantially increase the probability of elevated wall shear stress on the curved vessel surface.

3.3.5 Effect of Capsule Shape and Sphericity

To further understand the impact of capsule shape on aggregation, simulations were conducted for spherical capsule suspensions flowing through cylindrical tubes, as illustrated in Fig. 3.13. Each spherical capsule's volume is set equal to that of a single red blood cell, and

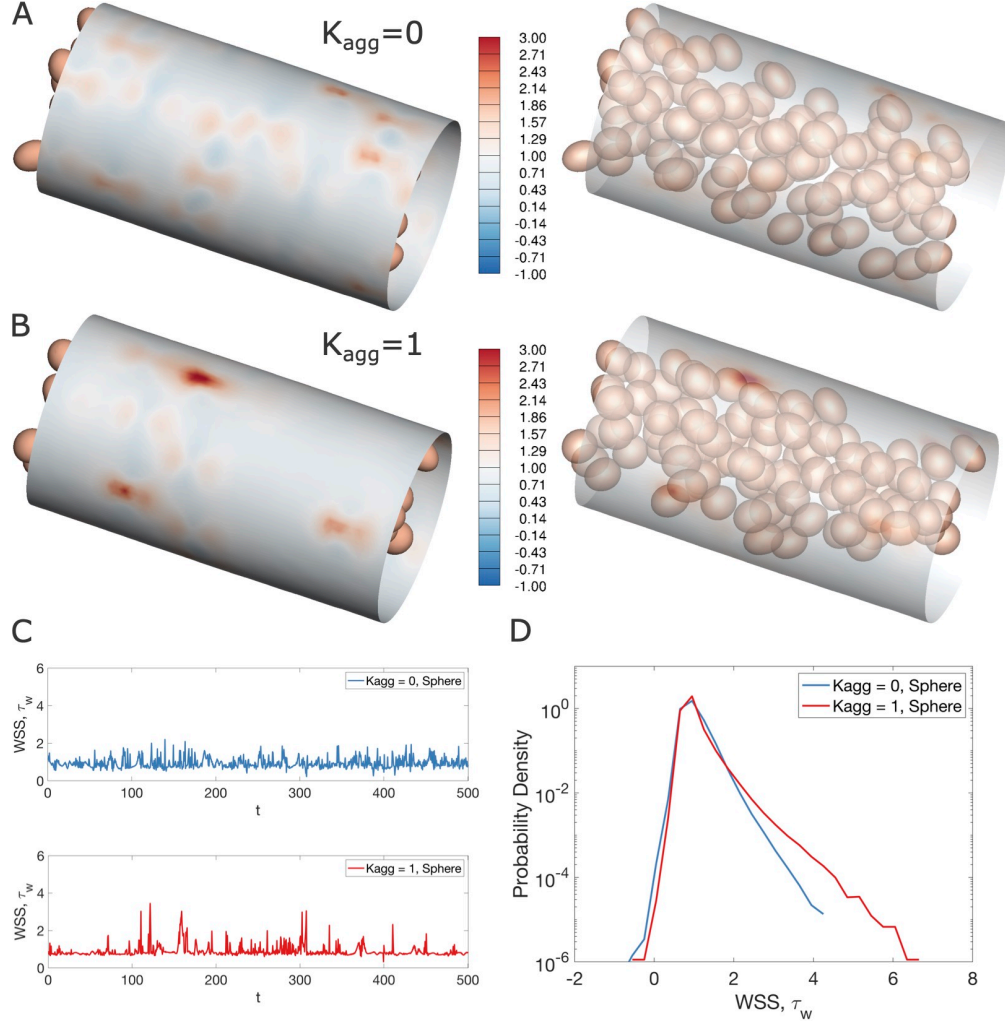


Figure 3.14: Simulation snapshots of (left) wall shear stress on the cylindrical surface and (right) corresponding transparent views in spherical capsule suspension with (A) $K_{agg} = 0$ and (B) $K_{agg} = 1$. (C) Temporal evolution of wall shear stress at a fixed point on the cylindrical surface in spherical capsule suspension. (D) Probability density profile of wall shear stress on the cylindrical surface.

the hematocrit remains fixed at 20%, ensuring the number density of capsules in the suspension remains constant. Analysis of the simulation snapshots in Fig. 3.13 reveals that in the absence of aggregation, the spherical capsules are relatively evenly dispersed throughout the tube. Conversely, in scenarios where aggregation occurs, the spherical capsules aggregate to form clusters.

Fig. 3.13 presents statistics regarding the distribution of cells in the tubes and the thickness of the cell-free layer. Fig. 3.13 (A) illustrates that in the absence of aggregation, the cell-free layer exhibits relatively uniform distribution, with an average thickness that is thin-

ner. Conversely, in the presence of aggregation, the probability profile of cell-free layer thickness becomes broader, with a heightened likelihood of very thin cell-free layers. Importantly, it is noteworthy that overall, the thickness of the cell-free layer in the spherical capsule solution is thicker compared to that of the red blood cell suspension, particularly evident in scenarios involving aggregation. This disparity arises because RBCs possess a larger surface area-to-volume ratio than spherical capsules, thereby facilitating intercellular aggregation and cluster formation. Consequently, the probability of encountering a very thin cell-free layer is higher in the red blood cell suspension. A parallel trend in cell distribution is also evident in the spherical capsule suspension, as depicted in Fig. 3.13 (B). In scenarios lacking aggregation, a pronounced peak is observed near the wall. However, in the case of aggregation, the spherical capsules are more distributed in the center of the channel.

3.4 Conclusion

This study uses direct simulations to examine the hydrodynamic effects of RBC aggregation on the shear stress environment in small blood vessels. RBC aggregation leads to a more heterogeneous spatial distribution of RBCs, leading to nonuniformity of the CFL thickness and increasing the probability of large shear stress fluctuations. local wall shear stress fluctuations as cells approach the vessel wall. This effect aligns with experimental findings about increased variation of CFL thickness and endothelial disruption in erythrocyte aggregation, supporting the hypothesis that aggregation-induced proximity of RBC clusters to the wall may contribute to glycocalyx reduction and vascular endothelial damage[118].

With a simple model of sickle cell disease, where there is a small population of smaller and stiffer RBCs, we illustrate the the effects of aggregation on wall shear stress fluctuations are exacerbated by these aberrant cells. These cells marginate because of their altered size and stiffness, and in the absence of aggregation, a distinct peak in sickle cell concentration is observed near the wall. Aggregation increases the mean CFL thickness, enhancing seg-

regation and margination, and increases the likelihood of sickle cells approaching the wall due to CFL thickness fluctuations. Accordingly, the presence of both aggregation and aberrant stiff cells leads to a larger probability of large wall shear-stress fluctuations than either aggregation or aberrant cells individually.

Simulations of blood flow through serpentine vessels reveal that vascular geometry significantly influences RBC aggregation and clustering. In the presence of aggregation, RBCs form clusters that lead to an inhomogeneous spatial distribution and increased variability in CFL thickness, as in the straight-tube case. This clustering effect is further accentuated by curvature, which promotes RBC aggregation near the wall, resulting in localized wall shear stress fluctuations. When segmented by region, the inner, middle, and outer areas of the channel exhibit significant perturbations in CFL thickness due to aggregation, increasing the probability of very thin CFL regions and thus elevated wall stresses. In cases involving SCD, aggregation causes sickle cells to marginate closer to the wall, further intensifying wall stress fluctuations and increasing the risk of vascular damage and inflammation.

4

Red blood cell partitioning and segregation through vascular bifurcations in a model of sickle cell disease ¹

¹The text of this chapter is adapted from the publication by X. Cheng, C. Caruso, W. A. Lam, and M. D. Graham. *arXiv*, 2501.04017

The impact of cell segregation and margination in blood disorders on microcirculatory hemodynamics within bifurcated vessels are physiologically significant, yet poorly understood. This study presents a comprehensive computational investigation of red blood cell (RBC) suspension dynamics, with a focus on a model of sickle cell disease (SCD) as an example of a disorder associated with subpopulations of aberrant RBCs. The findings reveal how cell margination influences cellular partitioning and distributions as well as vessel wall shear stress (WSS) at vascular bifurcations. Normal RBCs, which migrate toward the channel center, exhibit the Zweifach-Fung effect, preferentially entering high-flow-rate branches. In contrast, sickle cells, which marginate near the vessel wall, demonstrate an *anti*-Zweifach-Fung effect, favoring lower-flow-rate branches due to their position within the cell-free layer (CFL). The upstream segregation of cells remains downstream through the bifurcation, where sickle cells accumulate along the outer branch walls. This accumulation of sickle cells increases the frequency of high WSS events via direct physical interactions, particularly on the outer side of high-velocity branches, potentially contributing to the vascular damage and endothelial disruption observed in many disorders that affect RBCs. In geometrically asymmetric bifurcations, cells preferentially enter branches with larger radii, underscoring the influence of geometric complexity on microcirculatory blood flow. These findings provide insights into microvascular hemodynamics in SCD and other blood disorders.

4.1 Introduction

The partitioning of blood plasma and cells near a vascular bifurcation is complex. In the microcirculation, when red blood cells (RBCs) flow through a bifurcating region of a blood vessel, they tend to preferentially flow into the daughter vessel with the higher flow rate. This phenomenon, called the Zweifach-Fung effect [229, 230], assumes a pivotal role in shaping the distribution of blood flow within the microvascular network. In blood disorders, some RBCs exhibit alterations in their physical properties due to pathological factors, render-

ing them stiffer and smaller than healthy RBCs, consequently promoting their margination towards blood vessel walls. Blood disorders frequently result in damage to the endothelial cells lining the blood vessel walls, potentially resulting in conditions such as stroke or atherosclerosis[1, 231]. Thus, there is a critical need to understand the flow behavior of RBCs in blood disorders and to understand the interplay between cell margination and complex geometries such as bifurcations. In this study, we employ detailed computational modeling to investigate microcirculatory dynamics and statistical distributions of blood cells near vascular bifurcations, with a focus on sickle cell disease (SCD). The analysis elucidates how cell segregation and margination influence the flow dynamics of cells through bifurcations, shaping the wall shear stress (WSS) environment within these vascular structures.

The spatial distribution of blood components is intricate, influenced by their physical properties such as shape, size, and deformability. In the microcirculation, normal RBCs migrate toward the vascular center, creating a cell-free layer (CFL) depleted of RBCs near the vessel walls. In contrast, white blood cells and platelets preferentially concentrate within the CFL, a flow-induced segregation phenomenon known as margination. Simulations by Kumar et al. [232, 233] revealed that stiffer capsules exhibit substantial margination, while softer capsules concentrate near the channel centerline. Similarly, Sinha et al. [110] investigated segregation in binary suspensions of spherical capsules, observing that variations in aspect ratio significantly influence their spatial distribution. These findings have been further corroborated through direct simulations of model blood cell suspensions[157, 158]. Consistent with the simulation results, a theoretical framework, based on cell-cell collisions and hydrodynamic migration [114, 159, 160, 200, 233], predicts the overall features of RBC suspensions, including the dependence of the CFL thickness on hematocrit and blood vessel size, and also indicates that in suspensions primarily composed of deformable RBCs, rigid cells will strongly concentrate near the walls during flow.

Aberrant RBCs arising from blood disorders often exhibit altered physical properties compared to healthy RBCs. A typical example is SCD, where abnormal sickle hemoglobin

polymerizes within RBCs under deoxygenated conditions, forming long fibers that disrupt cellular architecture [127]. This pathological process increases membrane stiffness and induces cellular dehydration, leading to reduced cell volume. Consequently, sickle RBCs become less deformable than normal cells, with certain subpopulations irreversibly adopting a characteristic sickle-like shape. In SCD RBC populations, Clavería et al. [155] observed a heterogeneous distribution, with low-density (less stiff) sickle cells preferentially remaining closer to the center of microfluidic channels, while the stiffest cells segregated toward the channel walls.

To investigate the role of cellular interactions in hematological diseases, Caruso et al. [1, 231] developed an *in vitro* microvasculature model composed of endothelial cells cultured on the inner surface of a microfluidic system. SCD RBCs were introduced into a suspension of normal RBCs and perfused through this endothelialized microfluidic system. The study revealed that the sickled cells marginate, and that VCAM-1, a biomarker of endothelial dysfunction, was upregulated in response to the flow of SCD RBCs compared to normal RBCs. These findings suggest that purely physical interactions between endothelial cells and SCD RBCs, facilitated by margination, are sufficient to trigger endothelial inflammation. Additionally, theoretical and computational studies have demonstrated that diseased cells marginate toward blood vessel walls due to their smaller size and increased stiffness, increasing the frequency of high WSS events [167, 198, 202, 231]. These observations underscore the potential for marginated aberrant cells to contribute greatly to the vasculopathy observed in various hematological disorders. Similar experimental and computational observations are found with iron deficiency anemia (IDA) [198], and a detailed computational study suggests that enhanced probability of high WSS events may be found broadly in disorders that generate a population of aberrant RBCs [202].

The partitioning of cells in blood flow through vascular bifurcations is primarily attributed to the nonuniform cross-sectional distribution of cells in the inlet vessel [28, 85]. Doyeux et al. [88] quantitatively elucidated the Zweifach-Fung effect, demonstrating that

particle enrichment in the high-flow-rate branch arises largely from the initial inlet distribution, which is determined by particle deformability and wall depletion effects [199]. Manoorkar et al. [234] investigated the flow of rigid particle suspensions through bifurcating channels, highlighting complex behaviors driven by uneven distributions caused by particle migration in the inlet branch. Using particle tracking velocimetry, Li et al. [235] analyzed particle transport and downstream concentrations in a successively branching vessel network. Similarly, *in vitro* experiments by Clavica et al. [236] confirmed the tendency of RBCs to preferentially enter the daughter branch with a higher flow rate. Building on this phenomenon, microfluidic devices have been developed to effectively separate and enrich blood components. For example, Yang et al. [237] designed a microfluidic device that efficiently separates plasma from blood cells by leveraging the Zweifach-Fung effect.

The hemodynamics of blood cells near vascular bifurcations have been extensively studied. Both *in vivo* experiments and computational simulations [238–243] have documented lingering and jamming effects on RBC partitioning, highlighting the influence of cellular membrane deformability, vascular geometry, and flow conditions. Direct numerical simulations have been instrumental in uncovering the dynamics of RBC suspensions in vascular networks. For example, Balogh et al. [244] identified cellular-scale mechanisms that govern partitioning dynamics in a model microvasculature, while Zhou et al. [245] revealed downstream RBC concentration heterogeneity upon altering the network’s outflow rates. WSS around bifurcations has also been a focus of investigation. Ye et al. [246] examined the effects of flow rate and bifurcation disturbances on the development of the CFL and WSS in branching vessels. Additionally, Balogh et al. [247] analyzed microvascular networks, revealing significant heterogeneity in WSS and WSS gradients, with the highest WSS occurring in precapillary bifurcations and capillaries.

Altered cellular physical properties, such as deformability and aggregation, significantly influence microvascular hemodynamics near bifurcations. Using a computational model of 3D blood flow in a capillary network, Ebrahimi et al. [175] demonstrated that increased RBC

stiffness alters RBC trafficking dynamics and elevates local WSS near vascular bifurcations. Rashidi et al. [248] experimentally examined the behavior of healthy and artificially stiffened RBCs in a microfluidic T-junction, revealing distinct focusing profiles in the feeding channel for rigid and healthy RBCs that dramatically impact the cell distribution in the successive daughter channels. In a related computational study, Ye et al. [249, 250] investigated the aggregation of multiple RBCs at microvessel bifurcations. Their findings showed that RBCs preferentially flow into the branch with higher RBC flux as cells are compactly arranged into rouleaux, which are difficult to break up at high hematocrit levels.

The margination of cellular components near vascular bifurcations has attracted more attention. In *in vitro* experiments, Sugihara-Seki et al. [251] investigated margination in microvessel blood flow using a Y-shaped microchannel, and explored the concentration profile of the margined particles in the daughter channels. Similarly, Bacher et al. [176] explored microparticle and platelet behavior at vessel confluences and bifurcations, identifying a demargination effect following confluences but not at bifurcations, which influenced platelet concentrations. Sun et al. [252] examined RBC-WBC interactions in 2D vascular networks digitized from normal and tumor tissues. Their findings revealed that aggregated RBCs interact more strongly with rolling WBCs, transmitting greater forces to the vessel wall through the WBC and amplifying stress fluctuations at bifurcation sites. These insights into WBC margination at bifurcations have inspired the design of microfluidic devices featuring bifurcations to efficiently separate cellular components [112, 253].

The study of cellular dynamics near vascular bifurcations is critically important for advancing knowledge of blood disorders. Enjalbert et al. [254] investigated the effects of tumor vessel compression on tissue oxygenation, uncovering abnormal RBC partitioning at bifurcations that contributes to tissue oxygen heterogeneity. In the context of tumor metastasis, the margination and adhesion of tumor cells play a critical role in determining the sites of extravasation. Wang et al. [255] simulated tumor cell behavior in a microvascular network, finding that tumor cells are more likely to extravasate at bifurcations in regions with

slow blood flow and moderate hematocrit levels. Similarly, Li et al. [256] computationally studied the motion of RBC suspensions in a Y-shaped microfluidic channel, comparing healthy and malaria-infected RBCs. Their findings revealed that the increased stiffness of malaria-infected RBCs causes them to preferentially enter the low-flow-rate daughter branch, a behavior attributed to their distinct initial distribution in the parent channel.

While numerous experiments and simulations have been conducted to investigate cell flow dynamics in the vicinity of bifurcations, there is a scarcity of studies specifically addressing the impact of margination on cell distribution, segregation, and wall shear stress at vascular bifurcations. In this study, we employ detailed computational modeling to investigate the dynamics of RBC suspensions in a simple model of sickle cell disease during flow through vascular bifurcations. Our focus encompasses the influence of cell margination on the distribution and partitioning of various cellular components, thereby affecting the stress exerted on the vessel surface. The phenomenon of margination gives rise to a distinct spatial distribution of cells across blood vessels, consequently leading to variations in the partitioning behavior and statistical distribution of different cellular components near bifurcations, and thus to alterations of the Zweifach-Fung effect in the microcirculation.

4.2 Formulation

We simulate a suspension of RBCs, modeled as deformable fluid-filled elastic capsules, flowing through bifurcated vascular geometries. We employ the computational model developed in our previous work; further details are available in [202]. The vascular system under consideration exhibits a radius ranging from 10 to $16\mu m$, with the bifurcating branches geometries conforming to physiological characteristics [257, 258]. The RBC suspensions in our simulations are binary mixtures containing both normal RBCs and aberrant RBCs associated with blood disorders such as sickle cells in SCD. The binary composition assumes a number fraction of 0.9 for normal RBCs and 0.1 for aberrant RBCs. This is a substantial simplification,

given that real blood cell populations in SCD exhibit a diverse distribution of cellular physical properties. A suspension of only normal RBCs, denoted as a healthy RBC suspension, is considered a control. The overall volume fraction near the inlet (tube hematocrit) is around 20%, consistent with the physiological value in the microcirculation [168, 169].

The distributions of cells and the cell-free layers near the inlet vessels are fully developed before entering the bifurcation region. To achieve this, our model incorporates the inflow and outflow boundary conditions for particulate suspensions, as detailed in [259]. This boundary condition is particularly well-suited for addressing complex vascular geometries that do not exhibit simple periodic boundary conditions. The implementation involves defining single-phase flow zones at both the inlet and outlet regions. Dirichlet velocity boundary conditions are enforced at the inlet and outlet to establish a Poiseuille flow profile and regulate the relative flow rates in each daughter branch. Downstream of the inlet single-phase zone, a particle introduction zone is established, while upstream of the outlet single-phase zone, a particle removal zone is introduced. Within the particle introduction zone, cells undergo periodic flow. Upon exiting the introduction zone, cells transition into the primary flow domain, at which point an identical cell is introduced at the entrance of the particle introduction zone. Similarly, cells nearing the outlet of the removal zone are continuously removed. This approach ensures that the total number of cells in the simulation is dynamic, eventually converging to a steady range. In addition, the sizes of the single-phase zones and the particle introduction/removal zones are designed to be large enough to facilitate fully developed flow conditions without affecting the flow condition near bifurcations.

The suspending fluid, blood plasma, is considered incompressible and Newtonian with a viscosity of about $\eta = 1.10 - 1.35\text{mPas}$. In this study, we assume a viscosity ratio of 1 between the intercellular matrix and plasma. While normal RBCs typically exhibit viscosity ratios of up to 15 [180], aberrant RBCs associated with various blood disorders can display even higher values. However, previous studies have demonstrated that the dynamics of individual cells [222] and cell suspensions [223] remain qualitatively robust across a broad

range of viscosity ratios. The discoid radius a for human RBC is about $4\mu\text{m}$. The RBC membrane in-plane shear elasticity modulus $G \sim 2.5 - 6\mu\text{N/m}$. In this study, a normal RBC is modeled as a flexible capsule with a spontaneous biconcave discoidal shape representing shear elasticity and an oblate spheroidal shape for bending elasticity [227, 228]. In the context of SCD, abnormal sickle hemoglobin polymerizes within RBCs upon deoxygenation, forming elongated fibers that disrupt the cellular architecture [127]. This pathological process increases membrane stiffness and induces cellular dehydration, thereby reducing the overall cell volume. Consequently, sickle RBCs exhibit significantly reduced deformability compared to normal cells, with certain subpopulations irreversibly adopting a sickle-like shape. In this study, a sickle cell is modeled as a stiff capsule with a curved prolate spheroidal rest shape, with a volume approximately 20% that of the normal RBC model. The deformability of a capsule in the pressure-driven flow is measured by the dimensionless capillary number $Ca = \eta\dot{\gamma}_w a/G$. This is set to be 1.0 for normal RBCs, which corresponds to $\dot{\gamma}_w \sim 1000 \text{ s}^{-1}$. The wall shear stress is dimensionless using $\dot{\gamma}_w \sim 1000 \text{ s}^{-1}$. Since aberrant RBCs in blood disorders are generally much smaller and stiffer than normal RBCs, the interfacial shear modulus G of the aberrant RBCs is assumed to be five times that of normal RBCs, which leads to Ca for aberrant RBCs being at most 0.2 times that of Ca for normal RBCs. All results are for simulations that have been run to a statistically stationary state.

In our simulation, we maintain a particle Reynolds number, denoted as $Re_p = \rho\dot{\gamma}wa^2/\eta$, at a fixed value of 0.1. Although this value of Reynolds number Re_p may be considered relatively high for microcirculation, it represents a compromise between computational accuracy and efficiency. To ensure the robustness of our findings, we performed verification using $Re = 0.05$ and observed no changes in our conclusions. The fluid is assumed to be incompressible and Newtonian, satisfying the Navier-Stokes equations. Employing a projection method facilitates the temporal advancement of the velocity field $\mathbf{u}$. The Chorin projection method is utilized to advance the velocity field $\mathbf{u}$. This method involves solving an advection-diffusion equation to determine the intermediate velocity field $\mathbf{u}^*$, followed by

solving a Poisson equation for pressure P to enforce the divergence-free. The bifurcated vessel is situated within a cuboidal computational domain of $30a \times 10a \times 28a$, and an Eulerian grid with dimensions of $300 \times 100 \times 280$ is employed. Fluid-structure interaction is addressed using the immersed boundary method (IBM). Specifically, our model incorporates two types of immersed boundaries: deformable moving cellular membranes and rigid nonmoving vascular walls. The capsule membrane is discretized into N_Δ piecewise flat triangular elements; $N_{\Delta p} = 1280$ for normal RBC, while $N_{\Delta t} = 682$ for sickle RBC. Differing values of N_Δ are selected to ensure comparable sizes of triangular elements with characteristic size $\sim 0.4\mu m$ on both capsules. We employ the “continuous forcing” IBM and “direct forcing” IBM methods for the RBC membranes and tube wall, respectively. The numerical methodology follows the approach delineated in previous works [178, 179, 202].

4.3 Results

4.3.1 Validation: Zweifach-Fung effect

We begin the description of results with a comparison between our model and experimental data on the Zweifach-Fung effect that is available in the literature [6]. Detailed validation results have been reported elsewhere [202]. To closely align with physiological parameters employed in experiments, we set the radius of the inlet vessel to $10\mu m$, as depicted in Fig. 4.1(A). To quantify fluid partition within the bifurcation, we introduce the parameter $\eta_Q = Q_{\text{left}}/(Q_{\text{left}} + Q_{\text{right}})$, representing the ratio of the volumetric flow rate at one daughter branch to that at the parent vessel, where Q_{left} and Q_{right} denote the volumetric flow rates in the left and right daughter branches, respectively. A higher value of η_Q signifies a preference for a greater fluid flow through the left branch. Similarly, the partition ratio for cell number flow rate, denoted as $\eta_N = N_{\text{left}}/(N_{\text{left}} + N_{\text{right}})$, is defined. As η_N increases, there is a discernible bias in cell flow toward the left branch as opposed to the right branch within the bifurcation.

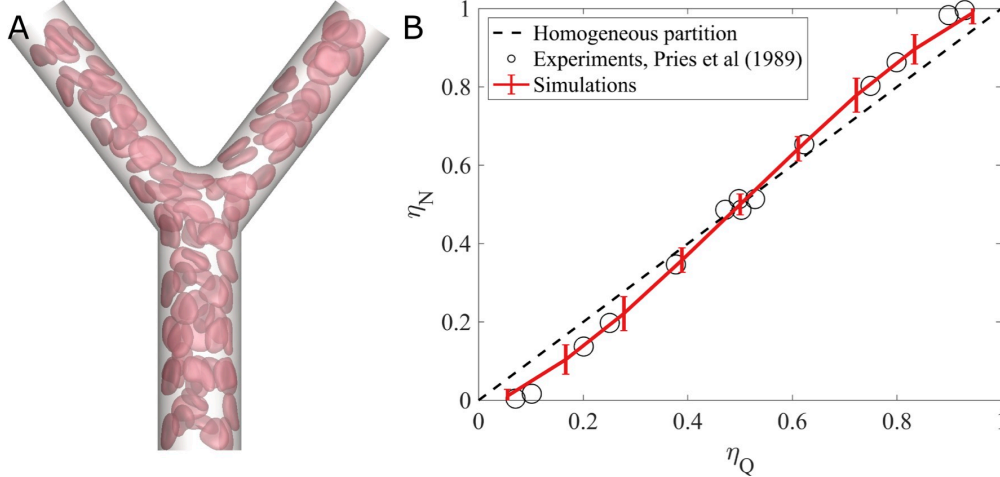


Figure 4.1: (A) Simulation snapshots of healthy RBCs partitioning flowing via a geometrically symmetric bifurcation. The radius of the inlet vessel is set to be $10\mu m$, while the outlet vessel radius is $8\mu m$. Cells flow from the bottom inlet to the top outlet branch. (B) Comparison of the Zweifach-Fung effect in our simulations with experimental results [6].

As depicted in Fig. 4.1(B), our simulation results exhibit good agreement with findings from the literature [6]. Specifically, a consistent pattern emerges wherein, when the left branch experiences a larger outlet volumetric flow rate than the right branch ($\eta_Q > 0.5$), the cell number partition ratio η_N exceeds the fluid partition ratio η_Q , and conversely when the right branch dominates in outlet flow, η_N is lower than η_Q .

4.3.2 Partitioning of Healthy and Aberrant Cells at a Geometrically Symmetric Bifurcation

We now characterize the Zweifach-Fung effect in the context of SCD, by considering a mixture suspension of normal RBCs with sickle RBCs flowing through a symmetric bifurcation. The radius of the parent and daughter vessels are set to be $16\mu m$ and $12.7\mu m$ respectively, and the angle between the two daughter branches is around 75° , as suggested by Murray's law [257] for bifurcating radii and Zamir's law [258] for bifurcation angles in the circulation.

As illustrated in Fig. 4.2(A), the fluid partition ratio, η_Q , is set to 0.9. The snapshot depicts the spatial distribution of normal and sickle cells in the vicinity of the bifurcation. Interestingly, a higher concentration of sickle cells is observed in the right branch, which

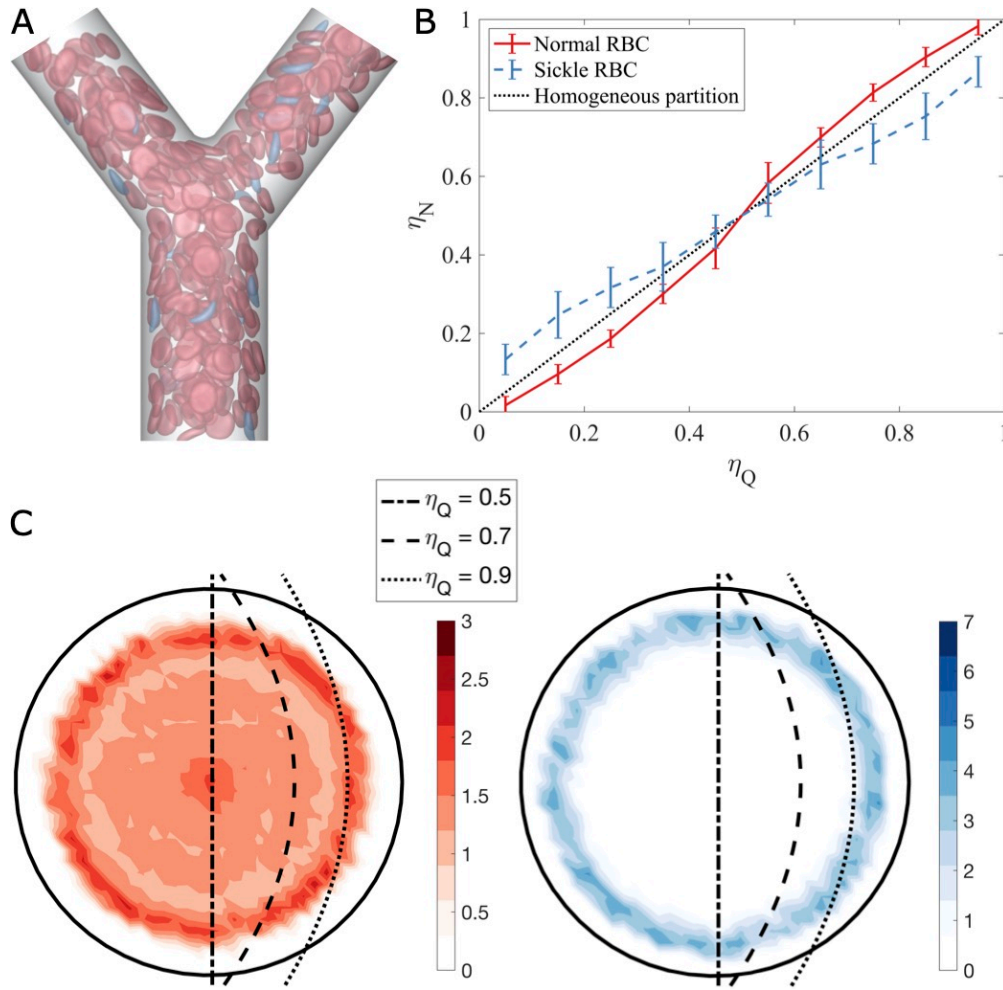


Figure 4.2: (A) Simulation snapshot of cell partition near a vascular bifurcation in sickle cell disease. Red particles are normal RBCs, while blue are sickle RBCs. Cells flow from bottom to top. The bifurcation exhibits geometric symmetry, characterized by a parent vessel radius of $16\mu\text{m}$ and daughter vessel radii of $12.7\mu\text{m}$. Despite this geometric symmetry, the fluid flow is asymmetric, with a fluid partition ratio of $\eta_Q = 0.9$. (B) Cell number partition ratio η_N as a function of volumetric flow partition ratio η_Q for normal and sickle RBCs in a suspension of SCD flowing through the geometrically symmetric bifurcation. (C) Cross-sectional cell number distribution for normal (red) and sickle cells (blue) before the bifurcation and the separatrix at various fluid partition ratios $\eta_Q = 0.5, 0.7, 0.9$. The color scale represents the cell number density of normal and sickle cells. In an ideal scenario where the cells are evenly distributed throughout the blood vessels, the cell density would uniformly equal 1 everywhere.

corresponds to the lower volumetric flow rate—a behavior that deviates from the classical Zweifach-Fung effect. Some early studies also documented deviations from the classical Zweifach-Fung effect under specific conditions. For example, Shen et al. [260] observed an inversion of the Zweifach-Fung effect in suspensions of less deformable RBCs at very low hematocrit, where the hematocrit in the lower flow-rate daughter branch exceeded that of the parent vessel.

To illustrate this more quantitatively, the variation of η_N relative to η_Q is depicted in Fig. 4.2(B) for normal and sickle cells, respectively. For normal cells in the mixture suspension, the Zweifach-Fung effect is again observed, in which normal RBCs tend to enter the higher flow rate branch preferentially. However, sickle cells do not conform to a similar partitioning behavior as observed in the Zweifach-Fung effect. Notably, at $\eta_Q > 0.5$, η_N for sickle cells is observed to be smaller than η_Q , and conversely at $\eta_Q < 0.5$. This indicates an inverse tendency in cell partition for sickle RBCs in comparison to normal RBCs; specifically, sickle RBCs demonstrate a preference for entering the branch with the lower flow rate as opposed to the higher flow rate branch; this is an *anti*-Zweifach-Fung effect.

To illustrate how the healthy and aberrant cells distribute at the bifurcation, Fig. 4.2(C) shows histograms of local hematocrit at a cross-sectional position well upstream of the bifurcation along with “separatrices” indicating which cells partition to the left and right branches for $\eta_Q = 0.5, 0.7, 0.9$. The separatrix is defined as the boundary line that determines the eventual flow trajectory of cells: cells located to the left of the separatrix are highly likely to flow into the left branch, while those on the right side are directed into the right branch. Separatrices are calculated and identified using polynomial logistic regression with a precision of around 0.97. Since sickle cells are primarily distributed near the blood vessel wall and insufficient data is available for the center of the channel, the cross-sectional position data of both normal and sickle cells is used to calculate the separatrices. Testing revealed that the separatrices accurately describe the partitioning of both normal and sickle cells, indicating that cell partitioning near the bifurcation is predominantly determined by their position.

In this figure we clearly see that the healthy cells exhibit a cell-free layer and a hematocrit profile that exhibits a ring of high hematocrit at the edge of the CFL and then another maximum at the centerline; this is consistent with past observations of cell or deformable capsule distributions [159, 200, 202, 232]. By contrast, the aberrant cells are strongly margined, residing in the cell-free layer. Under conditions of even partition ($\eta_Q = 0.5$), the separatrix is a straight line through the channel center, dividing the cross-sectional area into two equal segments. As η_Q increases, the separatrix gradually shifts and bows towards the right side. This shift plays a crucial role in how the position of the separatrix interacts with the uneven distribution of RBCs, giving rise to the Zweifach-Fung effect. In the conventional Zweifach-Fung effect, the shifts and bending of the separatrix toward the right results in the right region encompassing a much larger fraction of the CFL compared to the left. Consequently, a greater proportion of the cell-free plasma is directed into the right branch, reducing the normal cell number partition ratio, η_N , relative to η_Q . In contrast, the opposite trend is observed for margined sickle cells primarily located within the CFL. Consequently, these sickle cells are preferentially directed toward the right branch with a lower flow rate.

4.3.3 Cell Distribution and Segregation Downstream of Bifurcation

To characterize the distribution of cells after the flow bifurcation, Fig. 4.3 shows the cross-sectional distributions of number density downstream of the bifurcation, averaged over the “regions of interest” (ROI) indicated in the figure. This study examines the influence of bifurcation geometry on downstream cell distribution and segregation. Under uniform partitioning conditions ($\eta_Q = 0.5$), the cross-sectional cell number density in the left and right daughter branches reveals persistent segregation between normal and sickle cells: normal cells predominantly occupy the center of the branches, while sickle cells accumulate near the vessel walls. Notably, downstream of the bifurcation, sickle cells are concentrated along the walls, with a higher density observed on the outer side (away from the bifurcation cen-

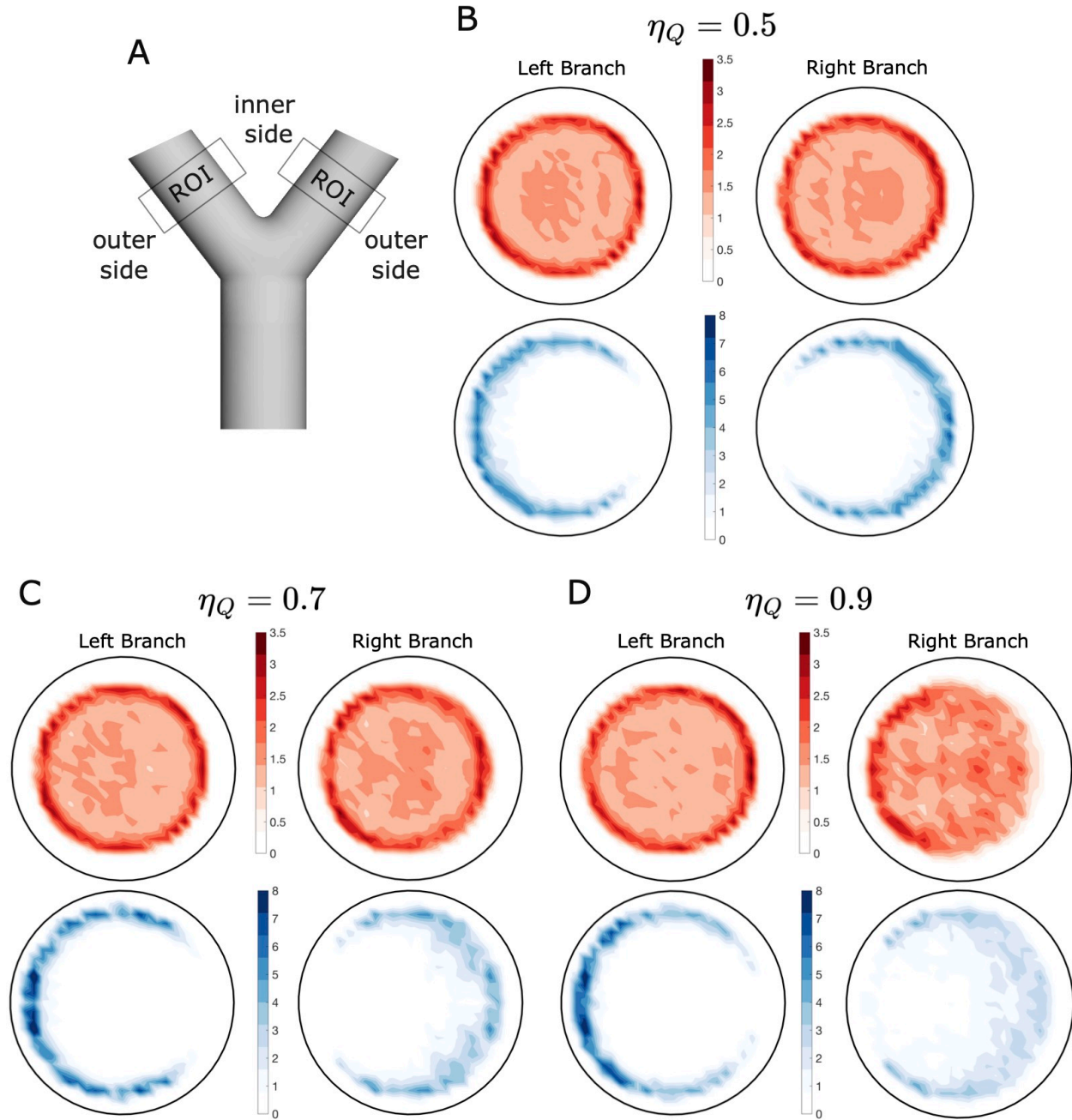


Figure 4.3: (A) Schematic showing the region of interest (ROI) near the bifurcation; Cross-sectional cell number distribution profile for normal (red) and sickle cells (blue) in the left and right branches downstream the bifurcation at various fluid partition ratios (B) $\eta_Q = 0.5$, (C) $\eta_Q = 0.7$, and (D) $\eta_Q = 0.9$. The color scale represents the cell number density of normal and sickle cells. In an ideal scenario where the cells are evenly distributed throughout the blood vessels, the cell density would uniformly equal 1 everywhere.

ter) and an absence on the inner side (closer to the bifurcation center). When partitioning becomes biased ($\eta_Q > 0.5$), increasing η_Q leads to higher flow velocities in the left branch and reduced velocities in the right branch. At $\eta_Q = 0.7$, the distribution of normal RBCs remains largely unaffected, while sickle cells in the left branch exhibit greater concentration near the outer wall, and their distribution in the right branch becomes less concentrated. At $\eta_Q = 0.9$, this trend becomes more pronounced, with normal RBCs in the right branch being biased towards the inner side wall. The cell-free layer on the outside of the right branch also becomes thicker. Simultaneously, the distribution of sickle cells in the right branch becomes less concentrated, due to fewer normal cells on the outside of the right branch.

4.3.4 Wall Shear Stress on Bifurcated Vessel

We have seen that the stiffer aberrant cells exhibit a pronounced tendency to marginate, resulting in close approaches between these RBCs and the blood vessel walls (endothelium). Experimental observations indicate that such interactions induce local fluctuations in wall shear stress, consequently triggering endothelial damage and inflammation [202]. To gain a comprehensive understanding of the hydrodynamic ramifications associated with cell margination and segregation at the vascular surface, we conduct an evaluation of the WSS near the bifurcation. As depicted in Fig. 4.4 (A), illustrating the transient distribution of WSS on the bifurcated vessel surface, distinct positive local WSS fluctuations are observed on the lateral side of the daughter branch, which is attributed to the proximity of sickle cells to the vessel wall, as evident in the corresponding transparent image.

For a detailed characterization of stress evolution over time at different locations on the vessel surface, we strategically selected specific fixed points ($a - f$) on the blood vessel surface of the bifurcated geometry, as illustrated in Fig. 4.4(B). The temporal evolution of WSS at these designated points was analyzed and compared between two suspensions: one containing only healthy RBCs and the other containing SCD RBCs. In Fig. 4.4(B), point a is situated at the center of the bifurcation, while points b , c , e , and f correspond to the

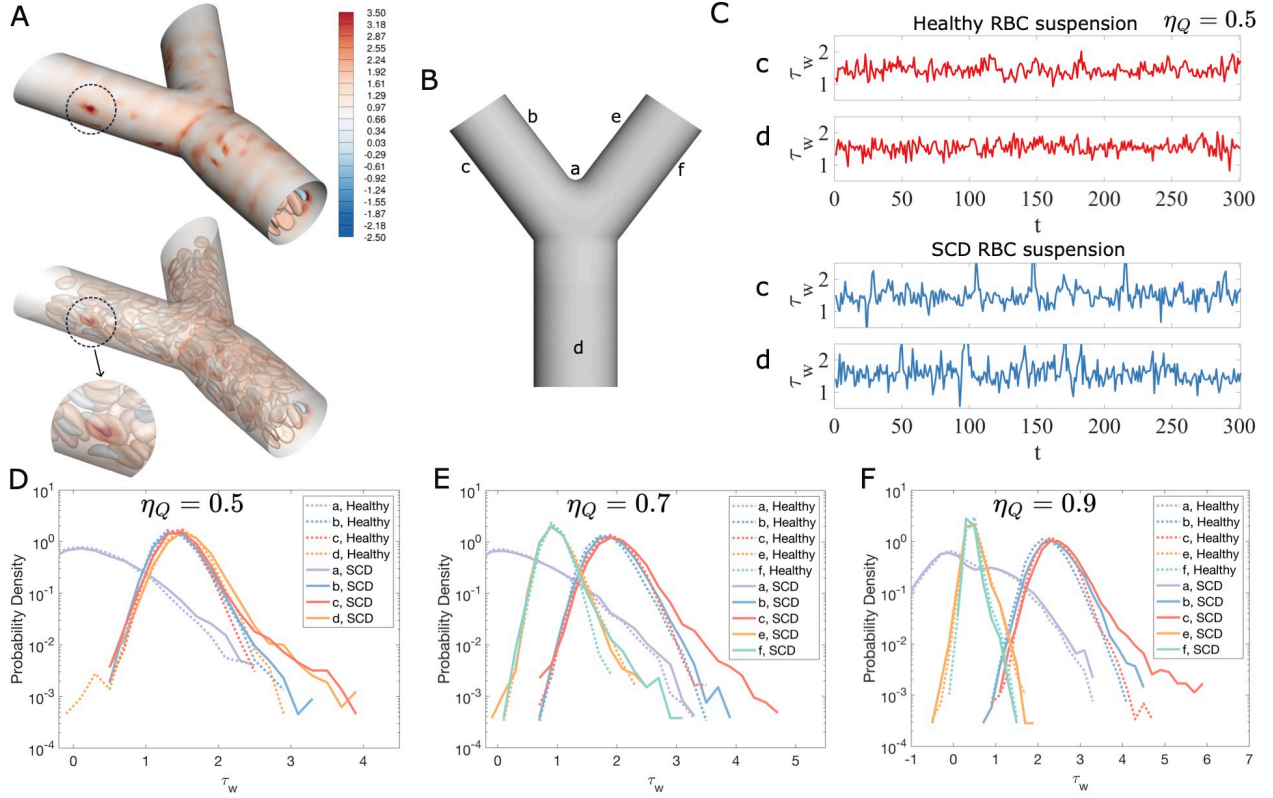


Figure 4.4: (A) Transient wall shear stress τ_w on (top) the bifurcation vascular surface and (bottom) corresponding transparent image. An area where a sickle cell close to the wall directly provokes WSS fluctuation in the daughter branch is indicated with a circle. (B) Schematic diagram of several sampling areas ($a - f$) on the surface of bifurcated blood vessels. (C) Temporal evolution of wall shear stress τ_w at selected fix points (c, d) on the bifurcation vascular surface with $\eta_Q = 0.5$. (D-F) Probability density profile of WSS τ_w at selected fix points on the bifurcation vascular surface with (D) $\eta_Q = 0.5$ (E) $\eta_Q = 0.7$, and (F) $\eta_Q = 0.9$.

inner and outer sides of the left and right sub-branches, respectively. In addition, point d represents a location distal to the bifurcation, closer to the inlet. A comparative analysis in Fig. 4.4(C) between the healthy and SCD RBC suspensions reveals large differences. The SCD suspension exhibits markedly larger WSS fluctuations, particularly at points c and d . Frequent and pronounced peaks are observed in the WSS temporal signal for the SCD suspension, whereas these peaks are absent in the healthy suspension. This disparity arises from the propensity of stiff, sickled cells to marginate and approach the vascular wall, inducing substantial local WSS fluctuations, as shown in Fig. 4.4(A).

To enhance statistical insight, Fig. 4.4(D) illustrates the probability density distribution of WSS under even flow partition ($\eta_Q = 0.5$) at selected points on the bifurcation surface for both healthy and SCD RBC suspensions. The distribution at point a is the broadest, aligning with the largest WSS fluctuations observed at this location. This is primarily due to the persistent presence of cells in proximity to the bifurcation. Despite these fluctuations, the average WSS at point a remains close to zero, owing to the geometric symmetry of the bifurcation. The presence of sickled cells notably increases the probability of encountering high WSS, particularly at points c and d , due to the tendency of stiff sickle cells to marginate toward the cell-free layer near the vessel wall and concentrate along the outer sides of daughter branches. This effect is less pronounced at point c , as fewer sickled cells are found on the inner side of the branch. Extending this analysis, Figs. 4.4(E, F) present WSS distributions under uneven flow partition ($\eta_Q > 0.5$), focusing on five points (a, b, c, e, f) on the bifurcation surface. The average WSS at points b and c exceeded that at e and f , reflecting the higher flow velocity in the left branch. Notably, the SCD RBC suspension exhibited a higher probability of elevated WSS, particularly at point c , located along the high-velocity branch, attributed to the accumulation of stiff sickled cells near the outer vessel wall. The WSS of the low-velocity branch is less sensitive to the presence of sickle cells, which is perhaps related to the observed reduced segregation. Overall, the margination and flow conditions within the bifurcation geometry influence the downstream distribution of sickle cells, leading to more large wall stress events on the outer side of the branch, where marginated sickle cells are more concentrated.

4.3.5 Geometrically Asymmetric Bifurcation

We now present results for an asymmetrical geometry, which is more representative of physiological conditions than the symmetric case considered above. To explore the impact of geometric asymmetry on cellular blood flow, we analyze the behavior of normal and sickle cells within an asymmetric bifurcation. As shown in Fig. 4.5(A), with η_Q set to 0.9 for the

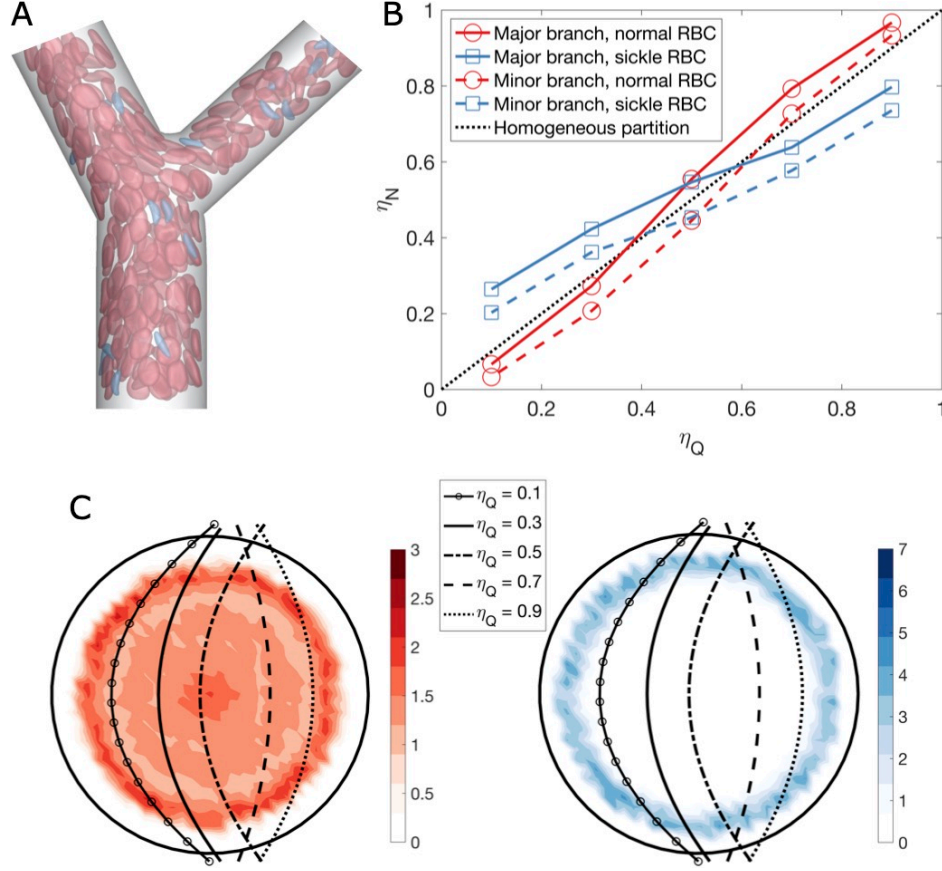


Figure 4.5: (A) A simulation snapshot of cell partition near a geometrically asymmetric vascular bifurcation in sickle cell diseases with $\eta_{Q, \text{major}} = 0.9$. Cells flow from the bottom inlet to the top outlet branch. In this asymmetric bifurcation geometry, the vessel radius at the entrance is $16 \mu m$, the left major branch has a radius of $14 \mu m$, and the right minor branch has a smaller radius of $11 \mu m$. (B) the cell number partition ratio η_N as a function of the volumetric flow partition ratio η_Q for normal RBCs (red) and sickle RBCs (blue). (C) Cross-sectional cell number distribution for normal (red) and sickle cells (blue) before the asymmetric bifurcation and the separatrix at various fluid partition ratios $\eta_{Q, \text{major}} = 0.1, 0.3, 0.5, 0.7, 0.9$.

left branch (larger radius), a greater accumulation of sickle cells and a thicker cell-free layer are observed in the right branch, which has a smaller radius and a lower volumetric flow rate. Note that the definition of partition ratio η in this figure is slightly different from the previous ones because of the introduction of geometric asymmetry. η_{major} and η_{minor} are defined for the major and minor branches, respectively. For instance, $\eta_{Q, \text{minor}} = Q_{\text{minor}} / (Q_{\text{major}} + Q_{\text{minor}})$. Fig. 4.5(B) depicts the relationship between η_N and η_Q for both cell types in the left (major) and right (minor) branches, revealing deviations from partitioning patterns observed in symmetric geometries. Even at $\eta_Q = 0.5$, cells exhibit a preference for the major branch, with

$\eta_N > 0.5$ for the larger-radius branch. This finding emphasizes the critical role of geometric asymmetry in cell distribution, favoring flow into the branch with a larger radius. Moreover, sickle cells again demonstrate the *anti-Zweifach-Fung* effect, exhibiting a tendency to flow preferentially into branches with lower volumetric flow rates.

To further investigate the impact of bifurcation asymmetry on cell partitioning behavior, Fig. 4.5(C) illustrates the separatrix at the entrance of the bifurcation. Under asymmetric conditions, even when the volumetric flow rates in the major and minor branches are equal ($\eta_Q = 0.5$), the separatrix is notably concave rather than straight. This curvature arises due to the larger radius of the left branch, resulting in $\eta_N > 0.5$ when $\eta_Q = 0.5$, which highlights a preferential flow of cells into the branch with the larger radius. The influence of geometric asymmetry on sickle cells is similar to that on normal cells; however, due to the proximity of margined sickle cells to the vessel wall and their tendency to stay within the cell-free layer, sickle cells continue to exhibit the *anti-Zweifach-Fung* effect. Overall, the geometric asymmetry of the bifurcation increases the curvature of the separatrix, further amplifying the uneven distribution of cells within the plasma.

4.4 Discussion

Our study first addresses the flow of cell suspensions through geometrically symmetric bifurcations, replicating the Zweifach-Fung effect, where cells preferentially flow into high-flow-rate branches. When analyzing a binary mixture modeling SCD blood, contrasting behaviors are found: while normal RBCs exhibit the expected Zweifach-Fung effect, stiff sickle cells demonstrate an *anti-Zweifach-Fung* effect, favoring lower-flow-rate branches. This arises because upstream of the bifurcation, normal RBCs are concentrated in the channel center, while margined sickle cells predominantly reside in the CFL near the vessel wall. Under symmetric geometric and flow partition conditions ($\eta_Q = 0.5$), the separatrix dividing upstream regions where healthy RBCs go to the left or right forms a straight line through

the channel center but shifts and curves as η_Q increases. This curvature leads to uneven plasma and cell partitioning, with relatively more cell-free plasma entering the branch with a lower volumetric flow rate. Thus, margined sickle cells, localized mainly within the CFL, exhibit an opposite trend to the Zweifach-Fung effect, underscoring the distinct partitioning behaviors of normal and aberrant cells in blood disorders.

This study also examines the influence of bifurcation geometry on downstream cell distribution. When a suspension of SCD cells flows through a bifurcated vessel, the segregation between cells observed upstream is preserved and remains downstream. Across a range of η_Q values, normal cells predominantly occupy the central region of the branch, while sickle cells accumulate in the cell-free layer near the vessel wall, particularly along the outer side of the branch. Furthermore, this study investigates the impact of SCD RBC suspensions on WSS in vascular bifurcations. Simulations reveal that margined stiff sickle cells exhibit increased physical interactions with the vessel wall, leading to pronounced local WSS fluctuations. Statistical analysis of WSS across key regions of the bifurcated vessel surface demonstrates that SCD suspensions greatly increase the probability of high WSS events compared to healthy cell suspensions, particularly near the outer branch wall where sickle cells preferentially accumulate downstream. This effect is further exacerbated in high-velocity branches under uneven flow conditions ($\eta_Q > 0.5$). These findings underscore the influence of cell segregation and flow environment in amplifying wall stress fluctuations, which potentially contribute to vascular and endothelial damage associated with sickle cell disease and other types of blood disorders.

Finally, the study explores the impact of geometric asymmetry in vessel bifurcations on cellular blood flow. In asymmetric geometries, where one branch has a larger radius, cells preferentially flow into the larger-radius branch, resulting in $\eta_N > 0.5$ even under equal volumetric flow conditions ($\eta_Q = 0.5$). Stiff sickle cells still display the anti-Zweifach-Fung effect, favoring branches with lower flow rates due to their margination and position in the cell-free layer. Under asymmetric conditions, the separatrix becomes more curved,

highlighting the critical role of vascular geometry complexity in shaping blood flow dynamics in the microcirculation.

5

Modeling cellular level blood flow in complex vascular geometries ¹

¹The text of this chapter is adapted from the publication by X. Cheng, C. Caruso, W. A. Lam, and M. D. Graham. *Science Advances*, 9.48, 2023

5.1 Methods

We have developed a computational model utilizing the immersed boundary method (IBM) to investigate cellular blood flow in complex vessel geometries. This approach offers the advantage of modeling flows in arbitrary geometries and accurately resolving the large deformation and dynamics of blood cells. Our model considers blood as a confined flowing suspension of red blood cells (RBCs) in the plasma. Two distinct types of boundaries are involved: deformable cellular membranes and rigid non-moving vascular walls with complex geometry. To handle these two types of interfaces, we use the continuous-forcing and direct-forcing immersed boundary methods, respectively. Specifically, the continuous-forcing IBM method couples surface stresses on the RBC membrane with the fluid flow, while the sharp-interface ghost-node immersed boundary method (GNIBM) is used to treat the rigid, non-moving vascular walls [178, 179]. The flow solver is based on a coupled finite-volume/spectral method, and our IBM code is parallelized using a hybrid MPI/OpenMP strategy.

5.1.1 Governing Equations

Assuming that blood plasma is both Newtonian and incompressible, the governing equations for its flow can be expressed as the incompressible Navier-Stokes equations.

$$\begin{aligned} Re \left(\frac{\partial \mathbf{u}}{\partial t} + \nabla \cdot \mathbf{u} \mathbf{u} \right) &= -\nabla P + \nabla^2 \mathbf{u} + \mathbf{F} \\ \nabla \cdot \mathbf{u} &= 0 \end{aligned} \tag{5.1}$$

5.1.2 Flow Solver

The Chorin projection method is utilized to advance the velocity field $\mathbf{u}$. This method involves solving an advection-diffusion equation (ADE) to determine the intermediate velocity field $\mathbf{u}^*$, followed by solving a Poisson equation for pressure P to enforce the divergence-free

constraints.

$$\begin{aligned}
Re \frac{\mathbf{u}^* - \mathbf{u}^n}{\Delta t} &= \nabla^2 \mathbf{u} - Re \nabla \cdot \mathbf{u} \mathbf{u} + \mathbf{F} \\
\nabla^2 P &= \frac{Re}{\Delta t} \nabla \cdot \mathbf{u}^* \\
\frac{\mathbf{u}^{n+1} - \mathbf{u}^*}{\Delta t} &= -\frac{1}{Re} \nabla P
\end{aligned} \tag{5.2}$$

For the ADE, we treat both the convection term and the body force term with the 2nd-order Adams-Bashforth method (AB2), and the diffusion term with the Crank-Nicholson method for numerical stability.

$$Re \frac{\mathbf{u}^* - \mathbf{u}^n}{\Delta t} = 0.5 \nabla^2 (\mathbf{u}^* + \mathbf{u}^n) + 1.5 N(\mathbf{u}^n) - 0.5 N(\mathbf{u}^{n-1}) + 1.5 \mathbf{F}^n - 0.5 \mathbf{F}^{n-1} \tag{5.3}$$

where $N(\mathbf{u}) = -Re \nabla \cdot \mathbf{u} \mathbf{u}$ denotes the nonlinear convection, evaluated then by the central differencing.

$$\begin{aligned}
N(u) &= -Re \left(\frac{\partial u u}{\partial x} + \frac{\partial v u}{\partial y} + \frac{\partial w u}{\partial z} \right) \\
N(v) &= -Re \left(\frac{\partial u v}{\partial x} + \frac{\partial v v}{\partial y} + \frac{\partial w v}{\partial z} \right) \\
N(w) &= -Re \left(\frac{\partial u w}{\partial x} + \frac{\partial v w}{\partial y} + \frac{\partial w w}{\partial z} \right)
\end{aligned} \tag{5.4}$$

To leverage the efficient inversion of tri-diagonal matrices, a Locally One-Dimensional (LOD) Alternating Direction Implicit (ADI) scheme is employed to solve the ADE. This scheme involves four steps: first, the explicit terms are handled; then, the x, y, and z directions are solved implicitly, one at a time.

$$\begin{aligned}
\frac{Re}{\Delta t} \mathbf{u}^{*/4} &= \frac{Re}{\Delta t} \mathbf{u}^n + 1.5 N(\mathbf{u}^n) - 0.5 N(\mathbf{u}^{n-1}) + 1.5 \mathbf{F}^n - 0.5 \mathbf{F}^{n-1} \\
\left(\frac{Re}{\Delta t} - \frac{1}{2} \frac{\partial^2}{\partial x^2} \right) \mathbf{u}^{**/4} &= \left(\frac{Re}{\Delta t} + \frac{1}{2} \frac{\partial^2}{\partial x^2} \right) \mathbf{u}^{*/4} \\
\left(\frac{Re}{\Delta t} - \frac{1}{2} \frac{\partial^2}{\partial y^2} \right) \mathbf{u}^{***/4} &= \left(\frac{Re}{\Delta t} + \frac{1}{2} \frac{\partial^2}{\partial y^2} \right) \mathbf{u}^{**/4} \\
\left(\frac{Re}{\Delta t} - \frac{1}{2} \frac{\partial^2}{\partial z^2} \right) \mathbf{u}^* &= \left(\frac{Re}{\Delta t} + \frac{1}{2} \frac{\partial^2}{\partial z^2} \right) \mathbf{u}^{***/4}
\end{aligned} \tag{5.5}$$

Therefore, the pressure field can be written as

$$P(x, y, z) = \sum_{m_1, m_2=0}^{N_x-1, N_y-1} e^{2\pi i \left(\frac{m_1 x_1}{L_x} + \frac{m_2 x_2}{L_y} \right)} \hat{P}_{m_1, m_2}(z) \quad (5.6)$$

Then the 3D Poisson equation in Fourier space is given by,

$$\begin{aligned} & -4 \left[\frac{\left(\sin \left(\frac{\pi \cdot \Delta x \cdot m_1}{L_x} \right) \right)^2}{\Delta x^2} + \frac{\left(\sin \left(\frac{\pi \cdot \Delta y \cdot m_2}{L_y} \right) \right)^2}{\Delta y^2} \right] \hat{P}_{m_1, m_2}(z) + \\ & \frac{1}{\Delta z^2} \hat{P}_{m_1, m_2}(z + \Delta z) - \frac{2}{\Delta z^2} \hat{P}_{m_1, m_2}(z) + \frac{1}{\Delta z^2} \hat{P}_{m_1, m_2}(z - \Delta z) = \widehat{RHS}_{m_1, m_2}(z) \end{aligned} \quad (5.7)$$

To solve the 3D pressure Poisson equation (PPE), we assume that the computational domain is periodic in two directions, namely x and y . We begin by performing a 2D fast Fourier transform (FFT) on each $x - y$ plane. Next, solve for the Fourier coefficients along the z direction. Finally, a 2D inverse fast Fourier transform (iFFT) is performed on each $x - y$ plane to obtain the pressure field.

5.1.3 Spatial Discretization

The IBM requires an Eulerian representation for the fluid flow and a Lagrangian representation for the immersed boundaries such as the RBC membrane. The governing equations are solved on the Eulerian grid. To avoid odd-even decoupling, spatial discretization is based on the staggered mesh, in which these scalar variables, such as pressure P , are located at the grid center, while vector variables, such as velocity u and force F , are located at the grid faces. All spatial derivatives are evaluated using second-order differencing. As for the Lagrangian mesh on the immersed boundaries, the membrane is discretized into piecewise flat triangular elements. An open-source software Gmsh [261] is used to generate the surface mesh for both vessels (Fig. 5.1) and various red blood cells (Fig. 5.2).

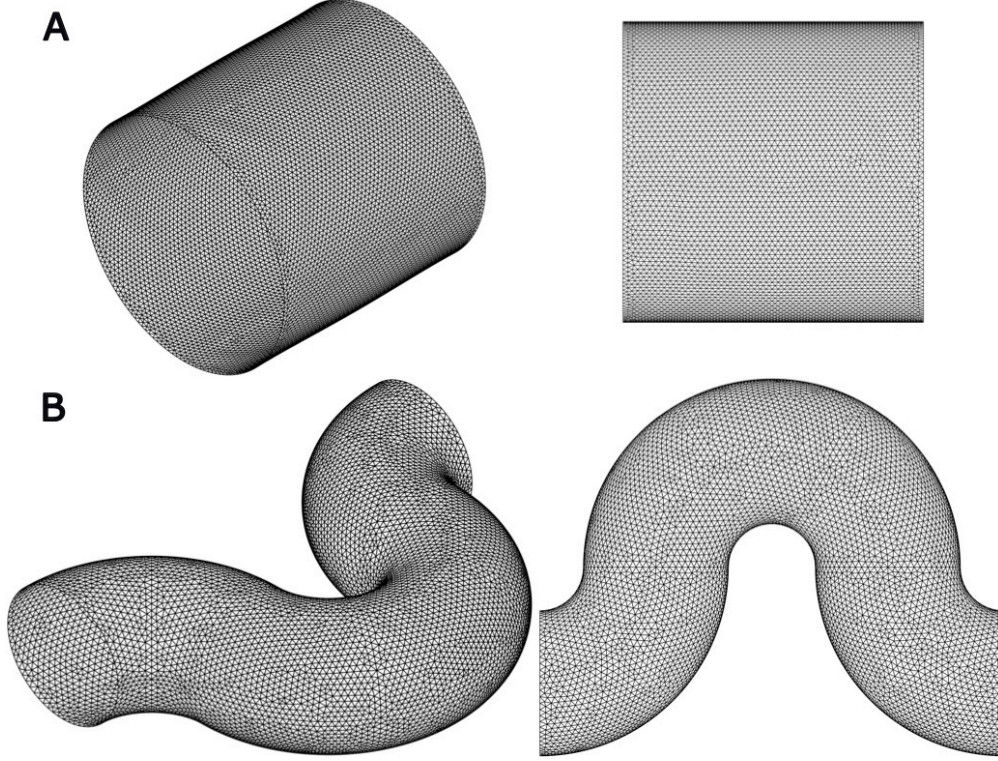


Figure 5.1: Surface mesh for (A) a straight cylindrical tube (radius $R = 20\mu m$ and length $L = 40\mu m$) and (B) a curved (serpentine) cylindrical channel (major radius $R_1 = 32\mu m$ and minor radius $R_2 = 20\mu m$).

5.1.4 Membrane Mechanics

The RBC membrane is assumed to resist shear deformation, area dilatation, volume conservation[262], and bending resistance. The total strain energy of the RBC membrane S is given by,

$$E = \frac{K_B}{2} \int_S (2\kappa_H + c_0)^2 dS + \overline{K_B} \int_S \kappa_G dS + \int_S W dS$$

where K_B and $\overline{K_B}$ are the bending moduli, and W is the shear strain energy density; κ_H, κ_G are the mean and Gaussian curvature of the surface, respectively; $c_0 = -2H_0$ is the spontaneous curvature, where H_0 is the mean curvature of the spontaneous shape. The first two terms correspond to the Canham-Helfrich bending energy [224, 225], and the third term comes from the shear strain energy stored in the RBC membrane. The strain energy density is computed based on the Skalak model [226]. A finite element method (FEM) is developed

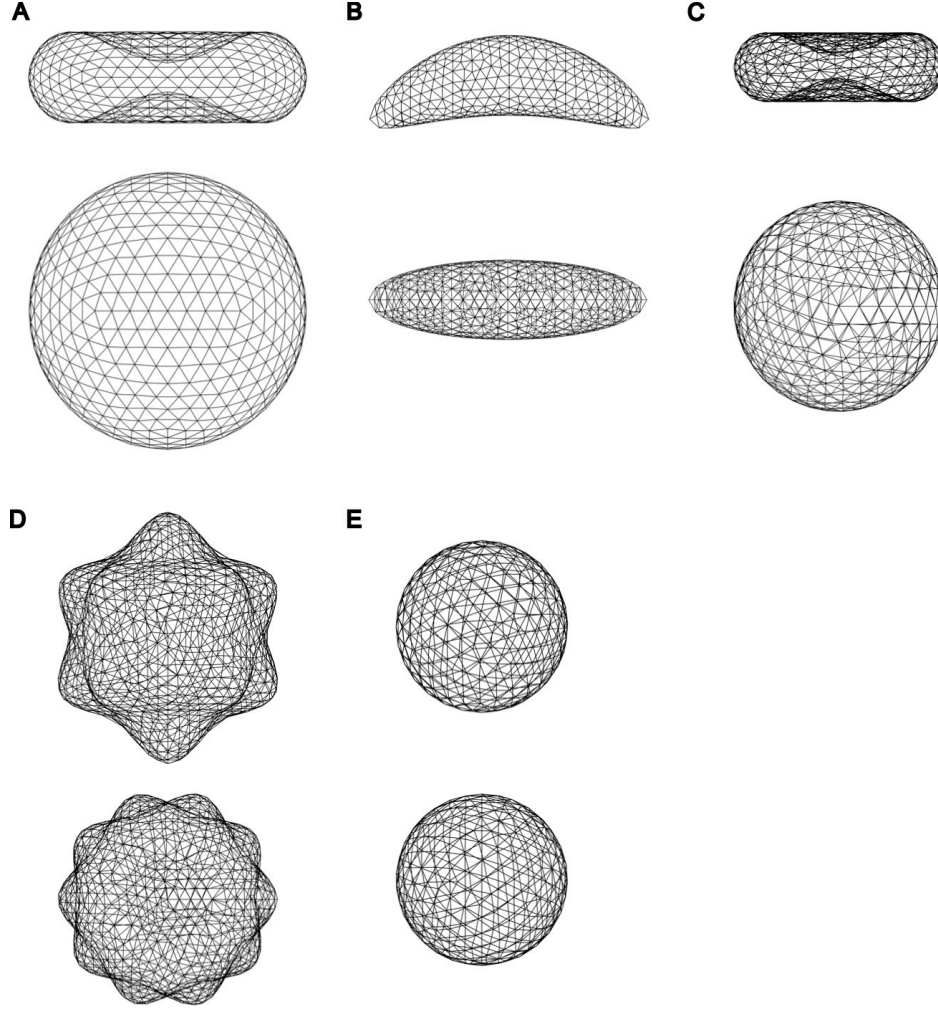


Figure 5.2: The surface mesh for capsules of (A) biconcave-discoid normal RBC, (B) sickle RBC, (C) iron deficiency RBC, (D) sphere-echinocyte, and (E) spherocyte. Note the size of each cell species in this figure is corresponding to the cell used in our simulation. The radius of normal RBC is $4\mu m$; aberrant RBCs are smaller than normal RBCs.

to find the surface stress as a result of membrane deformation [173].

5.1.5 Continuous Forcing IBM

The cellular membrane is deformed by the fluid flow, while the flow is altered by the membrane deformation in turn. This fluid-structure interaction (FSI) between flow and membrane is characterized via the continuous forcing IBM. The idea of this method is to add an external force term $\mathbf{F}$ to the right-hand side of the Navier-Stokes equation. The external force term originates from the membrane stress f_{membrane} . A discretized delta function is

used to spread the singular force on the membrane to the surrounding fluid and interpolate the fluid velocity back to the membrane.

$$\begin{aligned}\mathbf{F} &= \int_S \mathbf{f}_{\text{membrane}} \delta(\mathbf{x} - \mathbf{x}') d\mathbf{x}' \\ \mathbf{u}_{\text{membrane}} &= \int_{\mathcal{T}} \mathbf{u} \delta(\mathbf{x}' - \mathbf{x}) d\mathbf{x}\end{aligned}\tag{5.8}$$

δ is the three-dimensional Dirac-delta function, and $\mathbf{x}$ and $\mathbf{x}'$ are the locations in the flow domain $\mathcal{T}$ and on the cell surface S , respectively. A numerical approximation of the delta function is chosen to be

$$\delta(\mathbf{x} - \mathbf{x}') = \begin{cases} \frac{1}{64\Delta^3} \prod_{i=1}^3 \left[1 + \cos \frac{\pi}{2\Delta} (x_i - x'_i) \right], & |x_i - x'_i| \leq 2\Delta \\ 0, & |x_i - x'_i| > 2\Delta \end{cases}\tag{5.9}$$

where Δ is the Eulerian grid size.

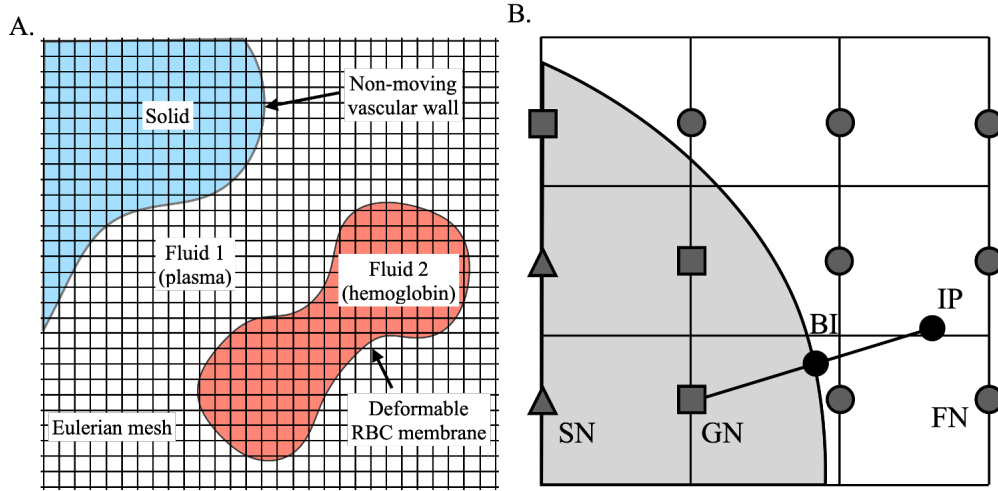


Figure 5.3: (A) Schematic describing of cellular-scale blood flow in complex geometry. This problem involves deformable RBC membrane and non-moving vascular walls as well as fluids of different viscosities. Boundaries are immersed in a fixed Eulerian mesh. (B) Schematic describing of sharp interface ghost-node immersed boundary method (GNIBM). BI: boundary intercept, IP: image point, FN: fluid node, SN: solid node, and GN: ghost node.

5.1.6 Direct Forcing IBM

Direct forcing IBM is used to treat another type of immersed boundary, the rigid, non-moving but geometrically complex vessel walls. Specifically, GNIBM is used to impose no-slip velocity boundary conditions on the vessel surface [179]. The idea of this method is to modify these differential operators appropriately to satisfy the no-slip boundaries condition on the geometrically complex vascular surface while maintaining second-order accuracy.

5.1.7 Indicator Function for Hematocrit Analysis

To characterize the cellular spatial distribution in RBC suspension, we define an indicator function $I(\mathbf{x}, t)$, such that the indicator function is one inside a cell, and zero outside a cell. It can be shown [263] that the indicator function $I(\mathbf{x}, t)$ follows a Poisson equation as

$$\nabla^2 I = \nabla \cdot \mathbf{G}, \quad \mathbf{G}(\mathbf{x}, t) = \int_S \delta(\mathbf{x} - \mathbf{x}') \mathbf{n} dS \quad (5.10)$$

where the $\mathbf{G}(\mathbf{x}, t)$ is an Eulerian variable constructed from the cell surface normals $\mathbf{n}$.

5.1.8 Wall Shear Stress Evaluation

To compute wall shear stress, the traction vector $\mathbf{t} = \boldsymbol{\tau} \cdot \mathbf{n}$ at the wall is determined using the velocity field approach outlined in [247, 264], where $\boldsymbol{\tau}$ is the stress tensor and $\mathbf{n}$ is the unit normal vector. The no-slip condition on the blood vessel surface yields the expression for the local wall shear stress $t_s = \mu \partial u_s / \partial r$. Note s denotes the local stream-wise direction. The second-order differencing method is utilized to numerically evaluate velocity derivatives.

5.2 Model Verification and Validation

5.2.1 Elastic Spherical Capsule in Simple Shear Flow

We consider a moving deformable spherical capsule subjected to simple shear flow, see Fig.5.4(A). The deformation of a capsule will cause stretching stress and bending stress on its surface. The deformability of a capsule is characterized by the nondimensional Capillary number $Ca = \mu\dot{\gamma}a/G$ and the nondimensional bending modulus $\hat{\kappa}_B = K_B/a^2G$, where μ is the fluid viscosity, $\dot{\gamma}$ is shear rate, a is the radius of the capsule, G is the shear elasticity modulus and K_B is the bending modulus. Larger Ca and $\hat{\kappa}_B$ means that a capsule is more flexible, while a stiffer capsule has smaller Ca and $\hat{\kappa}_B$. The deformation of the capsule is described by the Taylor shape parameter defined as $D_{xz} = (L - B)/(L + B)$, where L and B are the maximum and minimum radial distances of an ellipsoid with the same inertia tensor. In Fig.5.4(B), we show that steady state values of Taylor deformation parameter D as a function of dimensionless Capillary number Ca for different $\hat{\kappa}_B$. Good agreement is found between our numerical results and simulation results from previous literature [7].

5.2.2 Stationary Rigid Sphere in Simple Shear Flow

We consider a rigid stationary sphere subjected to a linear shear flow, which is given by $\mathbf{u}^\infty = [\dot{\gamma}z, 0, 0]$. The schematic of the model set is shown in Fig.5.5(A). The analytical solution for this problem is given by

$$\begin{aligned} u &= \frac{y\dot{\gamma}}{2} \left[1 - \left(\frac{r}{a} \right)^{-5} \right] + \frac{y\dot{\gamma}}{2} \left[1 - \left(\frac{r}{a} \right)^{-3} \right] - \frac{5}{2} \left(\frac{x}{a} \right)^2 y\dot{\gamma} \left[\left(\frac{r}{a} \right)^{-5} - \left(\frac{r}{a} \right)^{-7} \right] \\ v &= \frac{x\dot{\gamma}}{2} \left[1 - \left(\frac{r}{a} \right)^{-5} \right] - \frac{x\dot{\gamma}}{2} \left[1 - \left(\frac{r}{a} \right)^{-3} \right] - \frac{5}{2} \left(\frac{y}{a} \right)^2 x\dot{\gamma} \left[\left(\frac{r}{a} \right)^{-5} - \left(\frac{r}{a} \right)^{-7} \right] \\ w &= -\frac{5}{2} \frac{xyz\dot{\gamma}}{a^2} \left[\left(\frac{r}{a} \right)^{-5} - \left(\frac{r}{a} \right)^{-7} \right] \end{aligned} \quad (5.11)$$

where u, v, w are the velocity component in each dimension, $r = \sqrt{x^2 + y^2 + z^2}$ is the distance from a point in flow to the center of the sphere, a is the radius of the sphere. Using the

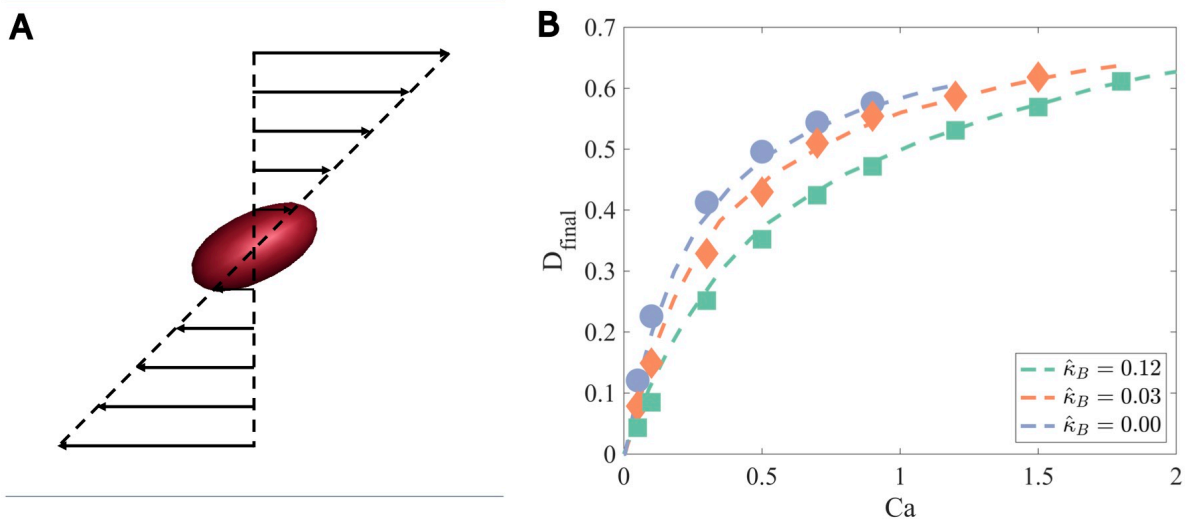


Figure 5.4: (A) Schematic diagram of an elastic spherical capsule in simple shear flow. Velocity Dirichlet boundary conditions are imposed on both the top and bottom plates to create a simple shear flow. Because of the existence of a constant shear rate, the sphere capsule is stretched and then deformed into an ellipsoid. (B) Steady-state Taylor deformation parameter for a spherical capsule as a function of Ca . Dash lines are results from previous literature [7], and symbols are our simulation results.

direct forcing IBM, we set the velocity on the sphere surface to zero (no-slip boundary condition). Note the solution above is for unbounded shear flow, thus we impose the Dirichlet velocity boundary conditions on both the upper and bottom walls and choose a larger computation domain to reduce the error by periodic boundaries. After the velocity field reaches its steady state, our numerical results are then compared with the analytical solutions. L_1 and L_2 error norms for different velocity components u, v, w are plotted in Fig.5.5(B). It is found that the direct forcing IBM in our model presents second-order accuracy.

5.2.3 Fåhræus-Lindqvist effect: Blood Relative Apparent Viscosity

Fåhræus and Lindqvist [81] made the noteworthy observation that the apparent viscosity at shear rates $\geq 100 \text{ s}^{-1}$, determined using Poiseuille's law in a capillary viscometer, exhibited a strong dependence on the capillary tube diameter. This phenomenon is called the Fåhræus-Lindqvist effect. For large tube diameters, a constant viscosity plateau was evident. But

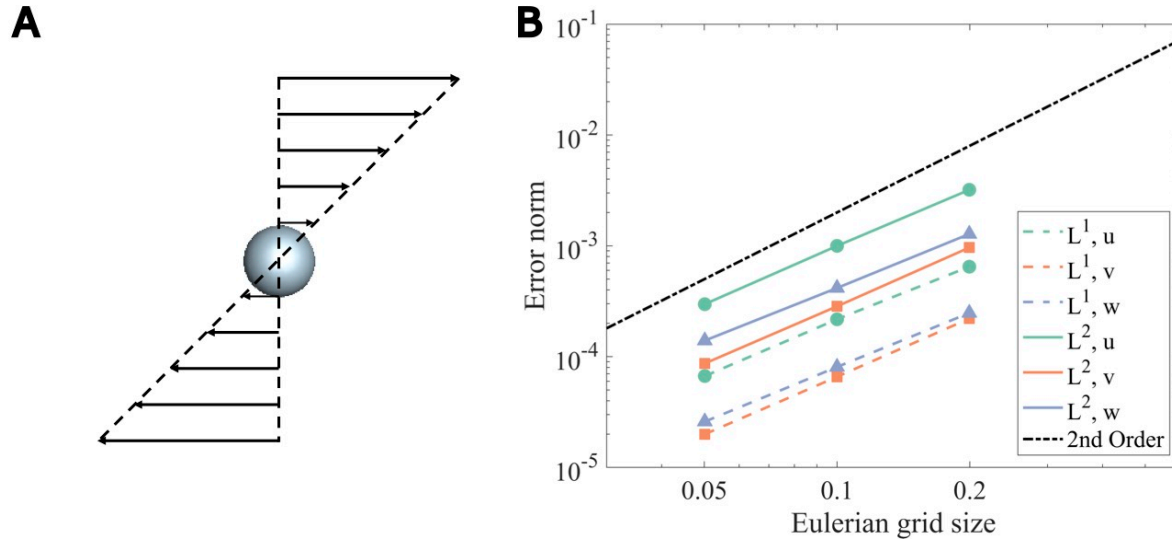


Figure 5.5: (A) Schematic of simulating a rigid stationary sphere subjected to a linear shear flow. The velocity field on the top and bottom wall is set to be the analytical solution (Dirichlet boundary condition). Periodic boundary conditions are imposed along x and y direction. A rigid sphere is placed at the center of the box. The velocity on the sphere surface is set to zero. (B) Error norm vs. mesh size for stationary rigid sphere in a linear shear flow.

within the range of 10 and $1000\mu\text{m}$, the apparent viscosity decreases substantially with decreasing tube size, before increasing sharply for tubes smaller than $10\mu\text{m}$. The increase for very small tubes is readily explained by the relative size of red blood cells, which are approximately $8\mu\text{m}$ in diameter, but the behavior at larger tube diameters is more subtle. In this study, we investigate the behavior of healthy RBCs flowing through tubes of varying radius, ranging from $R = 6\mu\text{m}$ to $R = 20\mu\text{m}$, as illustrated in Fig.5.6(A). Then the computed relative apparent viscosity, as a function of tube diameter, is shown in Fig.5.6(B). Fig.5.6 indicates that our numerical simulation qualitatively predicts the Fåhræus-Lindqvist effect. For comparison, we also show the empirical relation established by Pries et al [8] based on *in vitro* blood flow. It is found that our model predicts apparent viscosity in good agreement with the empirical relation at the physiological length scales we study in this work.

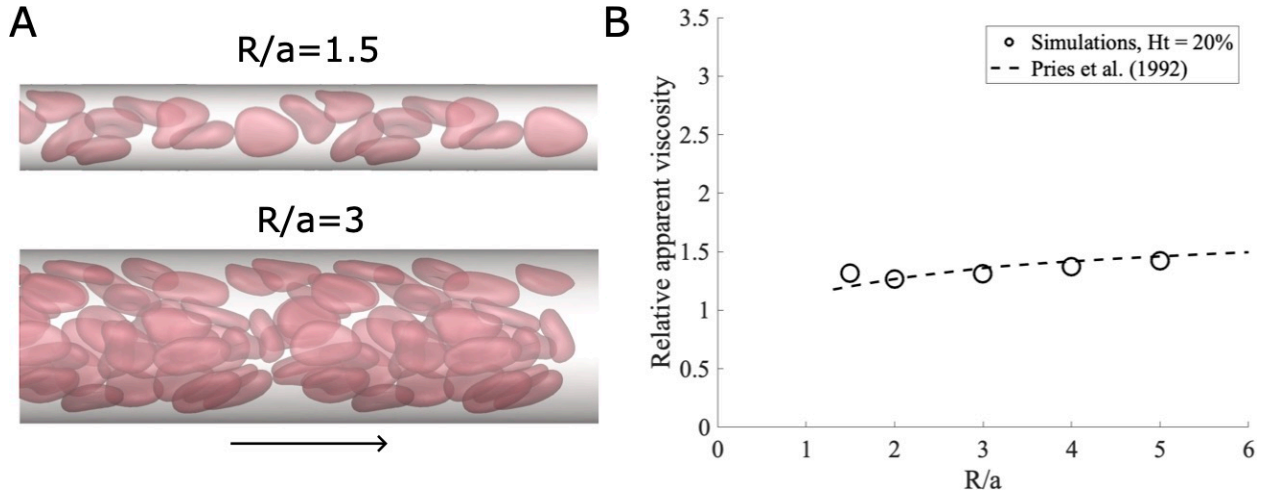


Figure 5.6: (A) Simulation snapshots of healthy RBCs with radius $a = 4\mu m$ flowing through tubes of various radius R . Hematocrit is set to be 20%. The arrow indicates the direction of flow. (B) The relative apparent viscosity as a function of tube radius R and comparison with experimental relation in [8].

5.2.4 Zweifach-Fung Effect: RBC Partitioning at a Bifurcation

The partitioning of blood plasma and cells near a vascular bifurcation is complex. In the microcirculation, when RBCs pass through a bifurcating region of a blood vessel, they exhibit a tendency to preferentially flow into the daughter vessel with a higher flow rate, resulting in fewer cells flowing into the vessel with a lower flow rate. This phenomenon, termed the Zweifach-Fung effect [229], plays a crucial role in shaping blood flow distribution within the microvascular network. Here we compare simulation results from our model with experimental observations from previous literature [6]. The radius of the inlet vessel is taken to be $10\mu m$, in order to be close to the value used in experiments, as shown in Fig.4.1. The Zweifach-Fung effect is quantified in Fig.4.1(B). The parameter η_Q is defined to characterize partition as the ratio of the volumetric flow rate at one daughter branch to the volumetric flow rate at the parent vessel. Similarly, η_N is defined as the ratio for cell number flow rate. From the results in Fig.4.1, it is found that our simulation results are in good agreement with the experimental findings [6].

5.3 Impact of Membrane Spontaneous Curvature on RBC Dynamics

Although efforts to understand RBC dynamics numerically have spanned the past two decades, the majority of these works have focused on RBC dynamics in cases where the RBC shape is symmetric across the shear plane or where the dimple is centered on the shear plane. Dupont et al.[265] demonstrated that an elastic capsule with a prolate spheroid rest shape, whose axis of symmetry is oriented off the shear plane, will exhibit a unique final dynamical motion for all initial orientations. Depending on the capillary number, they observed three final dynamical states: (i) rolling for lower capillary numbers, (ii) wobbling in which the capsule processes around the vorticity axis as the capillary number is increased, and (iii) a swinging-oscillating motion in which the long axis of the capsule oscillates around the shear plane with decreasing amplitude of oscillation as the capillary number increases, resulting in an in-plane swinging motion at high capillary numbers. Wang et al.[266] investigated the off-plane motion of oblate and prolate capsules and concluded that the final dynamical state could depend on the initial inclination angle. A recent study of RBCs in shear flow [267] has demonstrated that RBCs first tumble, then roll, transit to a rolling and tumbling stomatocyte, and finally attain polylobed shapes with increasing shear rate when the viscosity contrast between cytosol and blood plasma is large enough. Minetti et al. [268] give an exhaustive description of the dynamics under a shear flow of a large number of RBCs in a dilute regime is proposed. They identify which of the characteristic parameters of motion and of the transition thresholds depend on flow stress only or also on suspending fluid viscosity.

Similarly, Cordosco and Bagchi [269] studied the off-plane motion of oblate, prolate, and biconcave capsules. Unlike Dupont et al.[265] and Wang et al.[266], they included membrane bending stiffness in their formulation and considered a spatially uniform spontaneous curvature in the case of biconcave capsules. They found that rolling was the dominant mode in

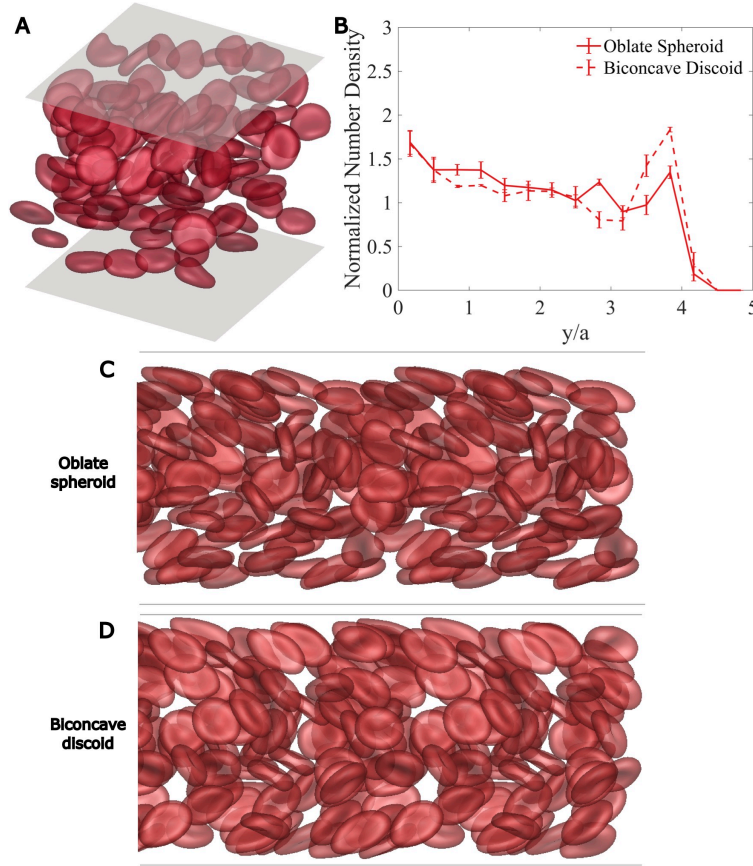


Figure 5.7: (Top) (A) Simulation diagram of homogeneous normal RBC suspension between the slit under pressure-driven flow. ($Re_p = 0.1$, Hematocrit = 0.15)(B) Steady-state wall-normal direction cell number density profile. Note here $y/a = 0$ denotes the slit center and $y/a = 5$ close to the wall. (Bottom) The side view of RBC suspension with bending spontaneous curvature being (C) oblate spheroid and (D) biconcave discoid.

the physiologically relevant viscosity ratio case (i.e., 5), tank-treading or wobbling mode at $\lambda < 1$, and an intermittent regime at low capillary numbers and low viscosity ratios, where the dynamics are dependent on the initial orientation. It is noteworthy that Bitbol[270] and Dupire et al.[265] experimentally observed rolling dynamics in a dextran solution where the viscosity ratio was less than unity. The discrepancy between simulation and experiment may result from the use of a spatially uniform spontaneous curvature that corresponds to a biconcave shape. It is important to note that to model the RBC membrane correctly, an assumption of the spontaneous shape has to be made, and finding the appropriate shape has been a challenge for both theoreticians and experimentalists. Sinha et al.[173] investigate

the cell dynamics' dependence on the membrane's spontaneous curvature. They found that an oblate spheroidal spontaneous curvature maintains the dimple of the RBC during tank-treading dynamics and exhibits off-shear-plane, tumbling consistent with the experimental observations of Dupire et al.[171]. For a complex structure such as an RBC membrane, it is possible that the natural shape for shear elasticity may differ from that for bending elasticity so the overall natural shape of an element results from the balance of bending and shear forces.

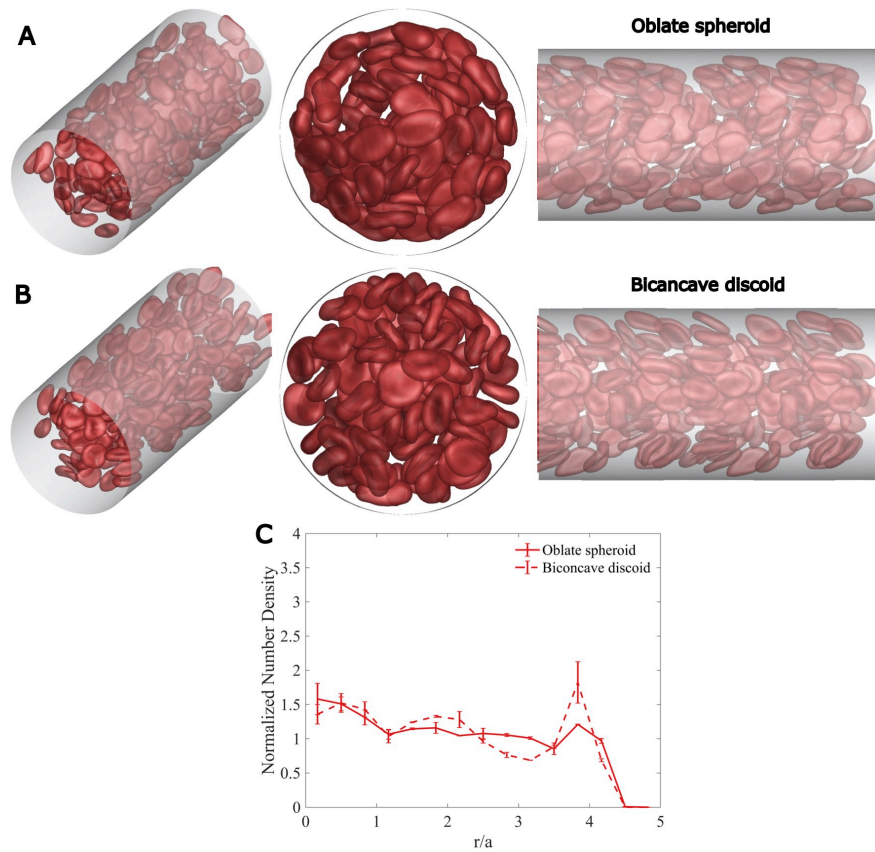


Figure 5.8: (Top) Simulation snapshots for homogeneous normal RBC suspension within the straight cylindrical tube with spontaneous bending curvature being (A) oblate spheroid and (B) biconcave discoid. ($Re_p=0.1$, Hematocrit = 0.20). (Bottom) (C) Steady-state radial cell number density profile for two types of RBC suspensions.

There have been endeavors to comprehend the impact of spontaneous shape on the ultimate dynamics of RBCs. Peng et al.[271] conducted a study on the influence of non-biconcave spontaneous shape on RBC dynamics and concluded that in order for an RBC to maintain

its biconcave shape during tank-treading, as noted by Dupire et al.[171], the spontaneous curvature must be non-biconcave. In instances where a biconcave spontaneous curvature was employed, tank-treading could not be achieved without significantly perturbing the initial shape. Additionally, Cordasco et al.[272] explored non-biconcave spontaneous shapes and ascertained that the spontaneous shape has a significant impact on cell dynamics, depending on the viscosity ratio. They observed that the dimple in the RBC remained intact for both biconcave and oblate spontaneous shapes. However, it should be noted that in both works, Peng et al.[271] and Cordasco et al.[272], non-biconcave spontaneous curvatures were investigated under the imposition of spatially uniform spontaneous curvature, denoted as c_0 . It is worth highlighting that RBC membranes differ from model lipid bilayers in that they possess embedded proteins with an underlying spectrin cytoskeleton and an asymmetric bilayer leaflet composition, all of which modify c_0 , with proteins in particular, having been demonstrated to preferentially bind via curvature-sensing mechanisms. Hence, it can be argued that c_0 would be spatially inhomogeneous.

Recently, using two different simulation techniques, Mauer et al. [273] construct a state diagram of RBC shapes and dynamics in shear flow as a function of shear rate and viscosity contrast [171, 274]. Their studies suggest that a nearly spherical stress-free shape best reproduces experimental results for the tumbling-to-tank-treading transition at low viscosity contrasts. Reichel et al.[275] combined simulation and experimental investigation of RBC shapes and dynamics in microchannels to provide a consistent RBC state diagram and illustrate the complexity of RBC behavior in the microflow. The RBC model employs a stress-free shape of the elastic spring network, corresponding to a spheroidal shape with a reduced volume of 0.96. Their simulation results agree well with experimental observations, allow the characterization of RBC variability in shear elasticity, and permit us to make a significant step toward quantitative measurements of RBC mechanical properties.

In our work, to investigate the effects of membrane curvature on the RBC dynamics, two types of homogeneous normal RBCs suspension are simulated: one with spontaneous

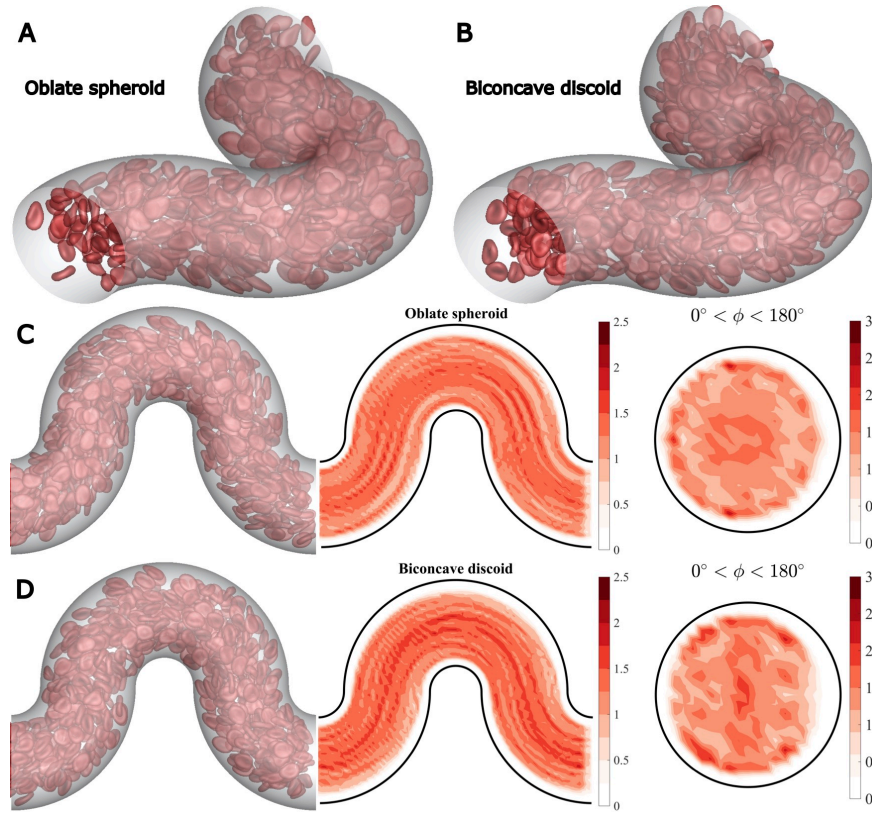


Figure 5.9: (Top) Simulation snapshots for normal RBC suspension in the curved serpentine channel with spontaneous curvature being (A) oblate spheroid and (B) biconcave discoid. (Bottom) Center-plane and cross-sectional cell number density distribution for RBC suspension with spontaneous curvature being (C) oblate spheroid and (D) biconcave discoid.

bending curvature being biconcave discoid, while another being oblate spheroid. Various vascular geometries are considered, including a slit (Fig. 5.7), a straight cylindrical tube (Fig. 5.8), and a curved (serpentine) channel (Fig. 5.9). The simulation snapshots tell that RBC rest shape has a nontrivial impact on its near-wall dynamics: RBCs with biconcave discoid rest shape tend to "rolling", while RBCs with oblate spheroid rest shape perform "tank-treading", consistent with the findings in a prior numerical investigation by Sinha et al. [173]. In spite of the change in orientational dynamics when the spontaneous shape is changed, no substantial change is observed in the number density distribution for normal RBCs.

6

Conclusions

6.1 General summary

This thesis investigates cellular-level blood flow in microcirculation under both healthy and pathological conditions, focusing on the distribution and dynamics of cellular components and their interactions with complex vascular geometries. Using detailed computational modeling validated by experimental data, it explores key phenomena such as cell segregation, margination, and altered intercellular interactions, including increased RBC aggregation in blood disorders. These studies reveal how microvascular geometries and pathological cell properties drive vascular dysfunction by disrupting hemodynamics, inducing endothelial damage, and altering wall shear stress. By providing new insights into the biomechanical mechanisms underlying vascular injury in conditions like sickle cell disease, this work establishes a strong foundation for future research in microvascular biomechanics and blood disorders.

In Chapter 1, we provide a comprehensive introduction to the composition and physiological functions of blood, with a focus on the differences in mechanics, quantity, and physiology among various cellular components. The microvascular system, including the endothelium and glycocalyx layer, is then discussed, emphasizing the complicated vascular geometry of the microcirculatory system and detailing the biochemical composition and mechanical properties of the glycocalyx layer. This chapter also summarizes the rheological properties of blood, particularly its behavior when the vessel size approaches that of RBCs. Furthermore, it introduces the segregation and margination of different cellular components during blood flow, reviews the contributions of our research group in this field, and clarifies the potential mechanisms underlying cell segregation and margination. The discussion extends to the biomechanics and endothelial damage observed in blood diseases, highlighting the pathological changes in blood cells associated with various hematologic conditions. Finally, this chapter summarizes key microfluidic observations and the role of endothelial damage in the progression of blood diseases, serving as a critical motivation for the work presented in this thesis.

In Chapter 2, we investigate the margination of aberrant cells in blood vessels and their interactions with the vessel wall, considering vascular geometry effects such as curvature. Blood disorders like SCD impact billions globally and are associated with vascular dysfunction and complications like stroke. While aberrant RBC properties and altered hemodynamics are well-studied, their contribution to vascular dysfunction remains unclear. Using a detailed computational blood model, we characterize cell distribution and vascular stresses in various RBC disorders, validated by experimental observations. The results show that smaller, stiffer aberrant RBCs marginate toward the vessel wall due to their physical properties. In curved channels, mimicking microcirculation geometry, cell distribution is highly heterogeneous. Marginated cells induce large transient stress fluctuations on the vessel wall, potentially contributing to vascular inflammation in RBC diseases.

In Chapter 3, we examine the impact of aberrant RBC aggregation on microcirculatory hemodynamics. Elevated RBC aggregation, observed in hematological disorders, is linked to endothelial injury and glycocalyx disruption, though the biophysical mechanisms are unclear. Using computational modeling, we analyze how RBC aggregation affects shear stress in small vessels, highlighting its role in vascular injury. RBC aggregation creates an inhomogeneous cell distribution, leading to variations in the CFL and fluctuations in wall shear stress near the vessel wall. These results align with experiments showing endothelial disruption near aggregated RBCs, which impair the glycocalyx. Power spectral density analysis identifies increased shear stress fluctuations due to aggregated RBC clusters near the wall. In blood disorders like SCD, aggregation-enhanced margination drives sickle cells closer to the vessel wall, amplifying shear stress fluctuations and increasing vascular damage risk. Curved vascular geometries intensify these effects by promoting RBC clustering near the wall, further elevating the likelihood of injury, particularly in SCD.

In Chapter 4, we investigate the impact of cell segregation and margination on microcirculatory hemodynamics in bifurcated vessels, focusing on SCD as a model of RBC disorders. Using computational modeling, we examine how cell margination affects cellular partition-

ing, distribution, and vessel wall shear stress (WSS) at vascular bifurcations. Normal RBCs, which migrate toward the channel center, exhibit the Zweifach-Fung effect, preferentially entering high-flow-rate branches. In contrast, sickle cells, which marginate near the vessel wall, demonstrate an *anti*-Zweifach-Fung effect, favoring lower-flow-rate branches due to their position in the CFL. This upstream segregation persists downstream, where sickle cells accumulate along outer branch walls, increasing high WSS events via direct interactions. These effects may contribute to vascular damage and endothelial disruption observed in RBC disorders. In asymmetric bifurcations, cells preferentially enter branches with larger radii, highlighting the role of geometric complexity in microvascular flow. These findings offer insights into microcirculatory dynamics in SCD and other blood disorders.

In Chapter 5, we discuss the computational modeling and implementation of microcirculatory blood flow in complex vascular geometries. We begin by introducing the numerical methods employed in this study, which include solvers for the governing flow equations and parallelization strategies, discretization techniques for blood cells and vessel walls, finite element methods for modeling membrane mechanics and cell deformation, immersed boundary methods for fluid-structure interaction, indicator functions developed for local hematocrit analysis, and approaches for calculating wall shear stress near the vessel wall. Subsequently, we both verify and validate the accuracy of the computational model. Verification is performed by comparing the model's results with analytical flow solutions, demonstrating that the error exhibits second-order convergence with decreasing mesh size. Validation is achieved by comparing the model's predictions with previously reported experimental results, showing strong consistency between simulations and observations. Finally, we analyze and verify the role of spontaneous curvature in the bending stress of the RBC membrane mechanical model. Our results indicate that an oblate spheroidal curvature provides a better fit to experimental data, offering improved accuracy in simulating RBC mechanics. This chapter establishes the robustness and reliability of the computational framework for studying microcirculatory blood flow in realistic and complex vascular geometries.

In the following sections, we explore potential future research directions, complemented by a review of relevant literature to contextualize and support these proposed avenues of investigation.

6.2 Future work

6.2.1 Models of Endothelial Glycocalyx and Its Interaction with Blood Cells

Early studies on flow through the endothelial glycocalyx layer (EGL) include Barry et al. [276], who analyzed steady and unsteady Newtonian fluid flows in a channel with a poroelastic wall. Wang and Parker [277] used mixture theory and lubrication theory to study a sphere moving through a fluid in a cylindrical tube with a deformable porous wall. Damiano et al. [278] extended these works by deriving solutions for axisymmetric pressure-driven flows with rigid particles in tubes lined by poroelastic materials. These studies modeled EGL flow using binary mixture theory, assuming a small solid volume fraction, which simplifies to the Brinkman equation for solenoidal velocity fields in the vanishing limit.

$$\begin{aligned} -\mu \nabla \times \nabla \times v - Kv &= \nabla p \\ \nabla \cdot v &= 0 \end{aligned}$$

The fluid velocity is denoted as $\mathbf{v}$, the pressure field as p , and the fluid's dynamic viscosity and hydraulic resistivity are represented by μ and K , respectively. These quantities are related through $K = \mu/k$, where k is the Darcy permeability. The dimensionless parameter $\delta^2 = \mu/(h^2K)$, where h is the thickness of the EGL, characterizes the relative balance between viscous forces and permeation-induced drag forces. For small values of δ , the flow is predominantly governed by Darcy flow, except in regions near the plasma interface. The penetration depth of axial flow into the EGL is given by $(\mu/K)^{1/2} = \delta h$. Given the small solid volume fraction of the EGL [279], its effective viscosity closely approximates that

of blood plasma ($\sim 0.01 \text{ dyn} \cdot \text{s}/\text{cm}^2$). The hydraulic resistivity, K , has been estimated from capillary recovery times following white blood cell passage and is reported to range from 10^{10} to $10^{11} \text{ dyn} \cdot \text{s}/\text{cm}^4$ [65, 67, 280]. For an EGL thickness of approximately $0.5 \mu\text{m}$, the corresponding values of δ^2 lie within the range of 10^{-4} to 10^{-3} . When the hydraulic resistivity K exceeds $10^{10} \text{ dyn} \cdot \text{s}/\text{cm}^4$, fluid flow within the EGL is significantly attenuated, effectively eliminating fluid shear stress at the surface of endothelial cells. This attenuation has profound implications for mechanotransduction, as the change of shear forces can alter the biochemical and biomechanical signaling pathways in endothelial cells.

There is common consent for modeling the fluid dynamical drag exerted by the EGL. However, hypotheses regarding the mechanisms responsible for its restoring force under physiological fluid shear stress (FSS) vary across different theoretical frameworks. Key models include the oncotic models proposed by Secomb *et al.* [48, 50, 51], the elastohydrodynamic models developed by Weinbaum *et al.* [65, 66], and the mechano-electrochemical model introduced by Damiano *et al.* [67, 280]. Pries *et al.* [281] hypothesized that oncotic pressure gradients created by plasma protein concentration exclude RBCs from the EGL region in capillaries. Secomb *et al.* [48] further posited that the restoration of the EGL following compression is chemically mediated, with oncotic pressure balancing matrix tension at equilibrium. Compression of the EGL increases the pressure difference, which relaxes the matrix tension, while recovery restores the equilibrium between tension and oncotic pressure. In their analysis, Secomb *et al.* [48] modeled single-file RBC motion in an EGL-lined capillary under the assumption that RBCs do not penetrate the EGL. The restoring forces acting on the red cell membrane were attributed to shear elasticity and isotropic tension, while flow through the EGL was described using the momentum equation developed by Damiano *et al.* [278]. An equilibrium oncotic pressure of $200 \text{ dyn}/\text{cm}^2$ (approximately 8% of plasma oncotic pressure) was determined to provide the traction force on the red cell membrane. Subsequently, Secomb *et al.* [51] refined their model to incorporate bending elasticity and investigated RBC invasion into the EGL at vanishing flow rates. Their findings indicated

that an oncotic pressure of 20 dyn/cm^2 was sufficient to exclude RBCs from the EGL region while allowing the red cell–capillary gap to approach zero [282].

The elastohydrodynamic model conceptualizes the core protein fibers of the EGL as possessing finite flexural rigidity, denoted by EI , which allows them to resist collapse under reduced oncotic pressure and withstand physiological FSS without significant deformation. This mechanical resistance has been corroborated through intravital microscopy observations [282, 283] and microviscometric studies of EGL thickness [63, 64]. Weinbaum *et al.* [65] proposed a linear elastohydrodynamic model to estimate EI , focusing on the phase of EGL restoration after finite compression caused by a passing WBC. They predicted a flexural rigidity of approximately $EI \approx 700 \text{ pN nm}^2$. Building on this framework, Han *et al.* [66] extended the model to include nonlinear elastohydrodynamic behavior by incorporating large deformation theory, a modified Brinkman equation, and local hydrodynamic resistance coefficients. This approach accounted for time-dependent forces and fluid imbibition during EGL expansion. Their experimental investigations detailed the temporal restoration of the EGL following WBC passage, extending earlier findings by Vink *et al.* [284]. Early theoretical studies on RBC deformation in capillaries often either neglected the presence of the EGL or assumed that RBCs did not penetrate it. However, experimental observations by Vink and Duling [282] revealed that a stationary RBC compresses the EGL to occupy the capillary lumen. Upon resumption of motion, the RBC exhibits a “pop-out” phenomenon, gradually rising through the EGL as its velocity increases. At a critical velocity, a thin lubricating layer forms between the RBC and the EGL, enabling smoother motion. For WBCs, interactions with the EGL involve both free motion and tethered rolling, depending on the forces acting on the tips of WBC microvilli and the penetration resistance offered by the EGL. These interactions are critical in understanding the dynamics of WBCs during inflammation and other physiological processes.

Early analyses of single-file RBC motion in capillaries lined with an EGL were conducted by Damiano [285] and Secomb *et al.* [48]. These studies demonstrated that a $0.5 \mu\text{m}$ -

thick EGL increases capillary resistance and reduces capillary tube hematocrit compared to smooth-walled glass tubes. The slower flow through the EGL necessitates larger RBC clearances and more elongated RBC shapes. Notably, these models were applicable to the high-velocity regime ($> 1000 \mu\text{m/s}$), where RBC deformation is maximized and becomes largely independent of flow velocity [286]. In contrast, RBC velocities in skeletal muscle capillaries typically range from 100 to $200 \mu\text{m/s}$ during steady flow and can drop to zero during intermittent flow. At these lower velocities, the balance between RBC membrane restoring forces, fluid shear stress, and pressure forces results in broader and shorter RBC shapes. To address the limitations of earlier models, Secomb *et al.* [51] refined their previous model [286] by incorporating RBC membrane bending elasticity, enabling analysis of RBC motion at velocities as low as $1 \mu\text{m/s}$. The refined model also examined the "pop-out" phenomenon, initially analyzed by Feng and Weinbaum [49], by investigating transient changes in RBC shape with velocity during a step increase in driving pressure. Feng and Weinbaum identified a significant fluid-dynamical lift force within the EGL, generated by plasma flow through the porous layer. This lift force depends on the presence of a confining boundary, such as the RBC membrane, moving at a small angle relative to the vessel wall, which prevents fluid leakage into the luminal space. Such conditions are observed when RBC motion resumes after flow cessation. Feng and Weinbaum emphasized that the dimensionless parameter $h/\sqrt{k}$, where h is the EGL thickness and k is the Darcy permeability, governs fluid motion. Using well-characterized mechanical properties of RBCs, Secomb *et al.* [51] quantitatively described the flow-dependent exclusion of RBCs from the EGL. Their work provided bounds on EGL mechanical properties and significantly advanced the understanding of hydrodynamic interactions between RBCs and the EGL.

The adhesion of leukocytes to vascular endothelial cells (ECs) during inflammation and immune surveillance begins with the tethering of L -, P -, and E -selectins, as well as α_4 integrins, to ligands on leukocyte microvilli or EC surfaces. While this process has been widely studied [287], many investigations neglect the role of the EGL or assume its compromise.

Despite its critical role in mechanotransduction and EC responses to FSS, the influence of the EGL on leukocyte adhesion and free rolling remains underexplored. A key question is whether WBC microvilli can penetrate the EGL to initiate tethered rolling [69]. This process is commonly observed in postcapillary venules, where an intact EGL compresses during WBC passage, bringing microvilli into close proximity with the EC surface [66, 284]. WBC microvilli, 0.3 to 0.7 μm in length [288], are comparable to the EGL thickness [63, 282], raising questions about free rolling through the EGL. Sedimentation forces on free-rolling WBCs (~ 0.3 pN) are amplified at microvilli tips due to viscous forces [69], enabling displacement of ≥ 500 nm from boundaries during rolling [289]. Zhao *et al.* [69] showed that contact forces increase with FSS, reaching 2 pN at 1 dyn/cm² and 5 pN at 10 dyn/cm² for equal-length microvilli, with higher forces for longer microvilli. These forces allow deeper EGL penetration during tethered rolling, with penetration depths reaching 200 nm at 1 dyn/cm² [69, 290]. At high FSS (> 10 dyn/cm²), penetration is minimal, explaining the rarity of tethered rolling. However, during tethered rolling in low-FSS regions, higher forces enable full EGL penetration, facilitating sustained interactions along postcapillary venules.

The EGL is critical in microvascular blood flow and cellular interactions. In some recent work, numerical simulations revealed the dynamic interplay between the EGL and RBCs, highlighting how EGL deformation, influenced by flow conditions and mechanical properties, significantly impacts RBC behavior and vascular flow resistance, particularly in microtubes of 7–10 μm diameters [291]. In addition, dissipative particle dynamics simulations explored the adhesion dynamics of nanocarriers to endothelial cells, demonstrating how ligand density, shape, and orientation of nanocarriers govern their penetration through the EGL. Entropy losses, particularly for hydrophobic ligands and shell structures, were found to be key barriers to efficient adhesion and penetration [292]. Moreover, investigations into capillary network formation from dispersed endothelial cells employed computational modeling to establish the conditions for spontaneous vascular network formation. The study identified ranges of traction forces, cell adhesion, and extracellular matrix rigidity required for robust network

morphology [293]. Furthermore, a study of electro-poroelastohydrodynamics in wavy-wall microvessels modeled pressure-driven flow of a non-Newtonian electrolyte through an EGL-covered channel. The results showed how viscoelastic fluid properties and induced streaming potentials depend on parameters such as the Weissenberg and Hartmann numbers, suggesting potential applications of the streaming potential as an energy source for medical devices [294].

Furthermore, Mirbod et al. developed a mixed analytical model of the feto-placental vascular system to analyze oxygen transport in human and mouse placentas. The model incorporated both conductive and terminal villi to predict optimal bifurcation levels, demonstrating close agreement with experimental results and providing new insights into the mechanisms of placental oxygen exchange [295]. Another study examined drag reduction in laminar flows over soft porous media, where a model was developed to describe Newtonian fluid flow over porous surfaces. The findings identified key parameters such as permeability and height ratios that govern drag reduction, with predictions validated by experimental data [296]. In an experimental investigation of particle migration in pressure-driven flows, Mirbod showed that porous geometries significantly affect particle concentration and velocity profiles, with concentrated suspensions forming high-concentration pathways through porous structures [297]. Further work on flow near random porous media used particle image velocimetry to measure slip velocity and penetration depth at the porous interface. The study highlighted the dependence of flow characteristics on porosity and extended theoretical predictions for penetration depth [298]. Finally, research into the stability of particle-laden flows in channels with porous walls demonstrated that wall permeability and particle characteristics play a crucial role in flow stability. The findings revealed strategies for controlling transitions between laminar and turbulent flows, particularly through the interplay of porous structures and particle properties [299].

As discussed previously, shear stress, the frictional force exerted by blood flow on vessel walls, is essential for vascular health, but pathological alterations can trigger inflammation through endothelial mechanotransduction. While traditional blood flow models treat blood

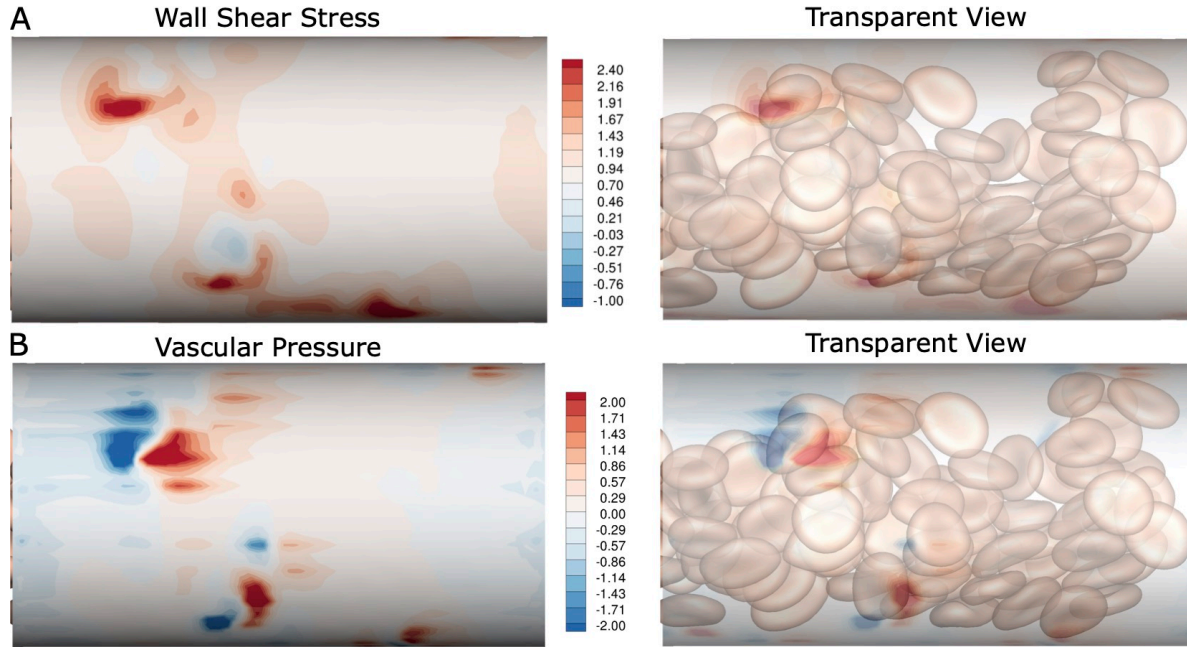


Figure 6.1: (Top) Wall shear stress and (bottom) pressure on the vascular surface in the flowing suspension of RBCs with aggregation strength $K_{agg} = 1$.

as a continuum fluid, the specific role of individual blood cells in circulatory shear stress remains poorly understood. Recent studies have linked altered RBC biophysics—such as decreased deformability, aberrant shape, and aggregation—in conditions like sickle cell disease, anemia, and severe COVID-19 to abnormal shear stresses and microvascular inflammation. Future work could focus on exploring how RBC mechanics directly induce endothelial dysfunction using computational modeling and endothelialized microfluidic systems. This includes developing multiscale computational models to investigate how RBC properties influence endothelial stress and dysfunction across various circulatory regions and examining endothelial mechanotransductive pathways triggered by pathological RBC mechanics through advanced experimental systems like live-cell rheometry and vasculature-on-chip. These efforts aim to provide deeper insights into hematological disorders, potentially paving the way for novel diagnostic and therapeutic strategies.

Here, we present some preliminary findings that extend our understanding of hemodynamic fluctuations associated with aberrant RBC aggregation. As demonstrated in the previous section, elevated levels of RBC aggregation result in substantial fluctuations in wall

shear stress along the vascular surface. Building upon this observation, we have computed and characterized the corresponding pressure fluctuations on the vascular surface. Guided by lubrication theory, which predicts local pressure variations when an object moves in close proximity to a surface, our analysis in Fig. 6.1 reveals that aberrant RBC aggregation significantly disrupts the uniformity of the CFL. Specifically, regions of very thin CFL thickness are associated with pronounced pressure fluctuations induced by RBCs in close proximity to the vascular wall. These findings provide new insights into the biophysical mechanisms underlying endothelial responses and the complex interplay between endothelial cells and blood cells.

6.2.2 Drug Carriers in the Blood Flow

The field of targeted drug delivery through the bloodstream has received significant attention in recent years due to its potential to revolutionize the treatment of various diseases. Targeted delivery systems have been explored for applications in cancer, cardiovascular, pulmonary, and inflammatory conditions [300–303]. Among these, cancer treatment remains the most extensively studied, as efficient drug delivery to tumors offers the potential to minimize drug dosages and reduce associated side effects [302, 303]. To achieve targeted drug delivery, a wide array of nanocarrier-based systems has been developed [304]. These nanocarriers are fabricated from diverse materials, including polymers (e.g., micelles, vesicles, dendrimers, nanogels), lipids (e.g., liposomes, micelles, niosomes), metals (e.g., iron oxide nanoparticles, gold nanoparticles, quantum dots), and carbon-based materials (e.g., carbon nanotubes) [302]. Beyond their composition, nanocarriers can be tailored in terms of shape, size, and surface modifications [304], allowing for customization to meet the requirements of specific applications. Tumor targeting by nanocarriers is achieved through two primary mechanisms: passive and active targeting [300]. Passive targeting leverages the enhanced permeability and retention (EPR) effect, which is a hallmark of tumor tissues characterized by leaky vasculature and impaired lymphatic drainage [300]. In contrast, active targeting

involves functionalizing the carrier surface with ligands, peptides, or counter-receptors that specifically bind to molecular markers expressed on the surface of tumor cells [305]. Regardless of the targeting approach, an effective drug delivery system must exhibit a strong propensity to marginate, enhancing its interactions with tumor vascular walls. As a result, optimizing the physical design parameters of drug delivery particles—such as their shape, size, and deformability—remains a critical area of ongoing research aimed at improving the efficiency of drug delivery systems.

The phenomenon of drug delivery particle margination has not been extensively studied, although recent investigations have begun to shed light on its underlying mechanisms. Charoenphol *et al.* [306] examined the binding efficiency of vascular-targeted spherical carriers using parallel-plate reconstituted blood flow assays. Their study explored the effect of particle size on adhesion efficiency and found that particle attachment to vessel walls decreased as the particle diameter was reduced from $10\text{ }\mu\text{m}$ to 100 nm under a shear rate of 200 s^{-1} . Notably, varying the hematocrit between $\phi = 0.3$ and $\phi = 0.5$ had little impact on the results. A key observation from this study was the presence of a critical wall shear rate at which particle adhesion was maximized. Adhesion decreased at shear rates both lower and higher than this critical value, with larger particles exhibiting a lower critical shear rate. The authors attributed the maximum adhesion to a balance between increased particle flux at higher shear rates and the disruptive effects of elevated shear stress on adhered particles. Based on their findings, Charoenphol *et al.* suggested that particles with diameters in the $2 - 5\text{ }\mu\text{m}$ range exhibit superior margination and wall adhesion at intermediate to high shear rates and channel heights compared to nanoscale particles. Tan *et al.* [307] further explored the role of RBCs in drug delivery particle distribution through numerical simulations. Their results revealed a nonuniform particle distribution, with a higher concentration near the vessel walls. This behavior was attributed to the tumbling motion of RBCs in the core region of the capillary, which enhances the radial dispersion of particles. Additionally, the volume exclusion effect of RBCs was proposed to play a significant role in facilitating the

margination of drug delivery particles toward the vessel walls.

Drug delivery via micro- and nano-particles has gained significant attention for its ability to enable targeted pharmaceutical transport. Margination, the migration of particles toward vessel walls, plays a crucial role in the adhesion of drug carriers. Müller et al. [308] used mesoscopic hydrodynamic simulations to study margination, showing that micron-sized ellipsoidal particles outperform sub-micron spherical particles due to their rotational dynamics near vessel walls. Cattaneo et al. [309] developed a computational framework to evaluate drug delivery efficacy, demonstrating that nanoparticle carriers address limitations of bolus injections in achieving optimal drug concentration in tumor tissues. Tan et al. [310] presented a numerical model using the lattice Boltzmann method to predict nanoparticle dispersion in red blood cell suspensions, showing that local disturbances from red blood cells significantly influence dispersion rates. Similarly, Coclite et al. [311] combined lattice Boltzmann and immersed boundary methods to analyze particle transport and adhesion, highlighting the role of particle shape in dynamics and adhesion under laminar flow. Ye et al. [312] introduced a multiscale computational approach to investigate magnetic nanoparticle margination in blood flow, revealing that external magnetic fields enhance particle margination and optimize accumulation in diseased tissues. Lastly, Bacher et al. [313] examined the clustering of microparticles in constricted blood flow, showing that marginated particles concentrate ahead of stenoses due to interactions with red blood cell plugs, potentially influencing microthrombus formation. Collectively, these studies underscore the importance of computational modeling in optimizing drug delivery and understanding particle dynamics in vascular environments. A promising direction for future work is to enhance the existing computational model by refining the immersed boundary method, incorporating stochasticity to simulate the Langevin dynamics of nanoparticles [314–316], and investigating the transport and dynamic behavior of nanodrug particles within blood vessels under blood flow. This approach aims to provide valuable insights for medical treatment applications.

In addition, some recent studies have advanced our understanding of nanoparticle trans-

port and drug delivery in blood flow, with significant implications for targeted therapies. Numerical simulations of hydromagnetic blood flow through stenosed arteries revealed that nanoparticles increase axial velocity, while radial magnetic fields dampen it, highlighting their potential for magnetic targeting in arterial diseases [317]. Investigations into particulate drug carriers showed that collisions, binding competition, and phagocytosis reduce leukocyte adhesion to inflamed endothelium, offering insights for improving drug efficacy in inflammatory conditions [318]. Modeling superparamagnetic nanoparticles in non-Newtonian blood flow demonstrated optimal particle capture in arterioles and capillaries, emphasizing the importance of magnet placement and release points [319]. Multiscale simulations of nanoparticle partitioning through microvascular bifurcations revealed RBC-entrainment effects driving heterogeneous distributions [259]. Finally, RBC hitchhiking was introduced, where nanocarriers adsorbed onto RBCs significantly improved delivery to the lungs and brain without toxicity, providing a versatile platform for clinical applications [320].

6.2.3 Thrombosis, Stenosis, and Other Vascular Geometries

Thrombotic diseases, including strokes and ischemic heart diseases, are leading causes of death and disability worldwide. Thrombus formation is a complex, multistep process involving biochemical reactions, mechanical stimuli, and hemodynamic forces. Recent research has focused on mechanical factors affecting thrombus formation, spanning scales from fluid dynamics to cellular biomechanics and molecular interactions [18]. However, replicating these intricate microenvironments remains challenging, both experimentally [29] and computationally [178, 321]. Macroscale studies have explored clot mechanics, blood rheology, and thrombus-fluid interactions using tools like rheometers and thromboelastography [322, 323]. Blood clots, broadly categorized as platelet-rich or erythrocyte-rich, exhibit distinct viscoelastic properties [324]. These properties alter blood flow by reducing velocity and increasing viscosity, causing abnormal shear stress fluctuations that further influence clot formation. Consequently, blood rheology is a critical biomarker of thrombosis [325, 326]. Al-

though macroscale techniques have advanced our understanding of thrombus formation, the underlying mechanisms often originate at the cellular and molecular levels [327]. Single-cell and single-molecule analyses, using tools like microfluidic devices, atomic force microscopy, and optical tweezers, have explored cell biomechanics, aggregation, adhesion, signaling regulation, and molecular mechanics [328–333]. At the cellular scale, platelet (PLT) adhesion and aggregation are critical to thrombus formation [14, 334, 335]. PLTs adhere to exposed proteins like collagen and von Willebrand factor (vWF) or are activated by soluble agonists such as ADP and thrombin, triggering cytoskeletal reorganization, spreading, and granule release. Biomechanical factors also play a key role; shear forces induce mechanosignals that convert mechanical stimuli into biological responses [336, 337]. Notably, platelets can be activated solely through biomechanical stimulation [338]. RBCs also influence thrombus formation by contributing to blood rheology and affecting platelet trajectories via intercellular interactions [339]. In some clots, RBCs dominate the composition, making their mechanical properties critical to thrombus mechanics.

Numerous simulation studies have aimed to reproduce thrombus formation, addressing aspects such as hydrodynamics, the coagulation cascade, PLT activation, cell mechanics, receptor-ligand adhesion, and fibrin network formation [340]. Pure-fluid simulations model blood as a homogeneous fluid, focusing on fluid field evolution around thrombi [341, 342]. Single-PLT simulations explore adhesion mechanisms by modeling individual platelets in fluid environments [343–345], while massive-PLT simulations investigate the collective behavior of rigid platelets, including aggregation, adhesion, and margination dynamics [346, 347]. Despite progress, integrating all these mechanisms into realistic 3D models remains challenging due to the complexity of combining diverse processes [321]. Macroscopic models struggle to incorporate detailed biochemical processes, while microscopic models face computational limitations when simulating fluid environments and large cell interactions. Thus, achieving a comprehensive numerical representation of thrombus formation remains an ongoing challenge.

Recent advancements in computational modeling have significantly enhanced our understanding of thrombosis formation and growth, offering insights into its biomechanics and clinical implications. Ye et al. [142] used a mesoscale particle-based method to show that high blood flow velocity effectively prevents microvessel blockage, surpassing the impact of antiplatelet or anticoagulant strategies. Zheng et al. [348] introduced a three-dimensional phase-field model coupled with particle-based simulations to predict thromboembolic risks under varying hemodynamic conditions, while Shankar et al. [349] developed a 3D multiscale model that accounts for platelet dynamics and thrombus morphology in realistic flow conditions, overcoming limitations of 2D models. Zhao et al. [350] refined computational fluid dynamics simulations to analyze hemodynamics in stenotic microfluidic models, highlighting stenosis as the dominant factor influencing shear stress and platelet behavior. Grande et al. [351] used patient-specific models to identify regions of high thrombotic risk in Kawasaki disease, emphasizing the role of agonist concentration and flow stagnation in pro-coagulant environments. Wu et al. [352] demonstrated the importance of vWF in shear-induced platelet activation and deposition in stenotic vessels. Collectively, these studies underscore the efficacy of computational models in predicting thrombotic risks, guiding treatment strategies, and informing the design of hemocompatible medical devices. Al Quddus et al. [353] used Smoothed Particle Hydrodynamics to model blood flow and thrombus formation, demonstrating good agreement with experiments under low Reynolds number conditions. Yang et al. [354] refined a macroscopic thrombosis model by incorporating ADP-mediated platelet activation, thrombus growth dynamics, and non-Newtonian blood flow, achieving improved alignment with experimental data. These studies highlight the versatility of computational models in advancing thrombosis research and guiding the design of hemocompatible devices.

Future research could focus on investigating blood flow in vascular geometries characteristic of conditions such as stenosis and microaneurysms, as shown in Fig. 6.2, building on existing computational models. Leveraging multi-scale simulation frameworks, such as those integrating platelet dynamics, shear-dependent thrombus growth, and biochemical activation

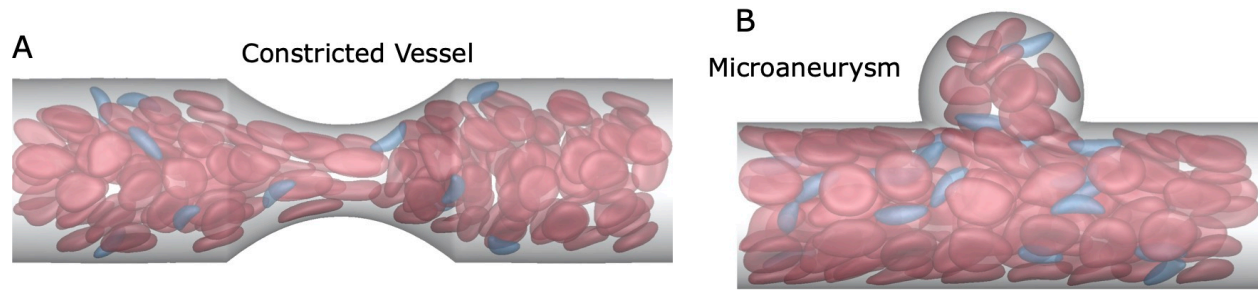


Figure 6.2: Simulation snapshots of SCD RBC suspension flowing through (A) constricted vessel and (B) microaneurysm. The flow direction is from left to right.

pathways, would enable a more comprehensive understanding of the mechanisms underlying hemostasis and thrombosis formation. Additionally, incorporating detailed analyses of cell-cell and cell-vessel adhesions, as well as the influence of non-Newtonian blood flow and agonist-mediated platelet activation, could provide deeper insights into the complex interplay of mechanical and biochemical factors driving these processes. Such advancements could pave the way for improved diagnostics and therapeutic strategies for thrombotic disorders.

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