Flow-induced segregation and dynamics of red blood cells in sickle cell disease

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Abstract

Blood flow in sickle cell disease (SCD) can substantially differ from the normal blood flow due to significant alterations in the physical properties of sickle red blood cells (RBCs). Chronic complications, such as inflammation of the endothelial cells lining blood vessel walls, are associated with SCD, for reasons that are unclear. In this work, detailed boundary integral simulations are performed to investigate the dynamics of a binary suspension of flexible biconcave discoidal fluid-filled capsules and stiff curved prolate capsules that represent healthy and sickle RBCs, respectively, subjected to a pressure-driven flow confined in a planar slit. The stiff component is dilute in the binary suspension. This system serves as an idealized model for blood flow in SCD. The key observation is that, unlike healthy RBCs that concentrate around the center of the channel and form an RBC-depleted layer (i.e. cell-free layer) next to the walls, sickle cells are largely drained from the bulk of the suspension and aggregate inside the cell-free layer, displaying a strong margination phenomenon. The marginated sickle cells are found to approximate a rigid-body-like rolling orbit near the walls. A binary suspension of flexible biconcave discoidal capsules and stiff straight (non-curved) prolate capsules is also considered for comparison, and we show that the curvature of the stiff component plays a minor role in the behavior of the binary suspension and the orbital dynamics of single cells. Additionally, by considering a mixture of flexible and stiff biconcave discoids, we reveal that rigidity difference by itself is sufficient to induce the segregation behavior in a binary suspension. Furthermore, the additional shear stress on the walls induced by the presence of capsules is computed for different suspensions. Compared to the small fluctuations in wall shear stress for the case with purely healthy RBCs, large local peaks in wall shear stress are observed for the binary suspensions, due to the proximity of the marginated stiff cells to the walls. This effect is most marked for the straight prolate capsules, which roll downstream very close to the walls. As endothelial cells are known to mechanotransduce physical forces such as aberrations in shear stress and convert them to physiological processes such as activation of inflammatory signals, these results may aid in understanding mechanisms for endothelial dysfunction associated with SCD.

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I. INTRODUCTION

Flowing suspensions of particles in confined domains, either homogeneous or heterogeneous, are of broad interest in a large variety of areas, with extensive applications in bioengineering and pharmaceutics using microfluidics. Typical examples include rapid separation of blood cells [1–3] and enhanced vascular-targeted drug delivery [4–9] involving blood flow in microfluidic systems as a model for human microvasculature. Blood, a classic archetype of multicomponent suspensions, is composed primarily of erythrocytes, or red blood cells (RBCs), with a hematocrit (i.e. volume fraction of RBCs) ranging from $0.1 - 0.3$ in the microcirculation to $0.4 - 0.5$ in large vessels such as arteries [10]. Two other major species of cells in blood, namely leukocytes, or white blood cells (WBCs), and platelets, are both considerably outnumbered by RBCs, by $\sim 500 - 1000 : 1$ and $12 - 32 : 1$, respectively [11]. In addition to the drastic difference in population density, these three cellular components exhibit distinct contrasts in physical properties such as shape, size, and rigidity [12]. Typically, RBCs are fluid-filled biconcave discoids with a radius of $\sim 3.8 - 4.0 \mu m$, WBCs are approximately spherical and slightly larger than RBCs, while platelets are the smallest. Healthy RBCs are highly flexible, while both WBCs and platelets are much stiffer. All these cellular components in blood are suspended in plasma. Under pathological conditions, however, diseased RBCs, such as those in patients suffering from sickle cell disease (SCD), often display substantial alterations in physical properties compared to healthy RBCs. These cellular-level alterations may lead to significant aberrance in the behavior of blood flow, and further cause complications. The insufficient understanding so far of both the dynamics of blood flow in SCD and the mechanism for complications associated with SCD, such as endothelial inflammation, has formed the principal motivation of the present work.

A significant feature of blood flow in the microcirculation, the network of microvessels (5 – 200 $\mu m$ in diameter [13]) consisting of arterioles, capillaries, and venules, is that all species of blood cells display a non-uniform distribution across the vessels. RBCs tend to migrate away from the vessel wall and form a RBC-depleted region termed cell-free layer next to the wall [14–18]. WBCs and platelets, in contrast, are found to reside near the vessel walls, a phenomenon known as margination [3, 15, 17, 19–21], which is of physiological significance as the prerequisite for inflammatory response and hemostasis. A large number of prior experiments [19, 20, 22–30], both in vivo and in vitro, and computational studies [21, 31–40]
have attempted to characterize the effects of a variety of parameters, such as shear rate, channel width, hematocrit, and RBC aggregation, on the strength of margination of WBCs and platelets. Furthermore, margination-based cell separation has been realized using lab-on-a-chip devices. For example, motivated by WBC margination in the microcirculation, Shevkoplyas et al. [3] achieved efficient separation of WBCs from whole blood using a simple network of microfluidic channels. Other examples include separation of stiffened malaria-infected RBCs from the healthy ones utilizing margination or lateral displacement of the stiff cells [1, 2]. Recent experiments by Fay et al. [24] showed that glucocorticoid and catecholamine hormones, such as dexamethasone and epinephrine, significantly reduce the stiffness of WBCs, which, as a result, attenuates WBC margination and leads to a substantial increase in the clinical WBC count.

The segregation behavior in blood flow, i.e., the discrepancy in cross-stream distribution propensity of different species of blood cells, substantially arises from their shape, size, and rigidity contrasts [41–43]. In a more general context, these factors, among other properties of the particles, are the determinants of the segregation behavior in an arbitrary flowing multicomponent suspension. A series of computational efforts have been made in the past years to examine the individual roles of various physical properties of the components in the segregation behavior. Kumar et al. [41, 42] numerically investigated the isolated role of rigidity difference in a binary suspension of neo-Hookean capsules subjected to either simple shear flow or pressure-driven flow in a planar slit. Here the rigidity (or deformability) of a capsule is characterized by the dimensionless capillary number, which represents the ratio of viscous stresses to elastic restoring stresses on the particle. They found that when the stiffer capsules are the dilute component, they display a substantial margination. In contrast, flexible capsules tend to enrich around the centerline of the channel when they are dilute, a behavior termed demargination. The effect of size contrast was also revealed [42]: in a mixture of large and small capsules with equal capillary number, the small capsules marginate while the large particles demarginate, in their respective dilute suspensions. Similarly, Sinha and Graham [43] explored the shape-mediated segregation behavior in binary suspensions of spherical and ellipsoidal capsules in simple shear flow. In this work, all capsules have the same capillary number, while the aspect ratio $\kappa$ of the ellipsoids is varied with either the equatorial radius or the volume of the capsules held constant; here $\kappa > 1$ and $\kappa < 1$ represent prolate and oblate spheroids, respectively. They showed that when the
ellipsoids have the same equatorial radius as the spheres, capsules with lower $\kappa$ marginate as the dilute component in a suspension containing primarily capsules with higher $\kappa$. When all capsules have the same volume, however, spherical capsules always marginate when they are dilute, while ellipsoidal (both oblate and prolate) capsules demarginate as the dilute component. In addition, these controlled studies [41–43] all revealed that the near-wall peak in the number density distribution profile for the marginated component shifts towards the wall as the volume fraction of the suspension increases, with a decrease in the cell-free layer thickness.

Inspired by all of the findings from the aforementioned experimental and computational investigations, a number of phenomenological theories have been developed aiming to provide insight into understanding the segregation behavior in flowing multicomponent suspensions of deformable particles. Most of the theories have been grounded on taking into account two key sources of cross-stream particle fluxes in flowing suspensions under confinement: wall-induced migration flux away from the wall, which is ubiquitous for any deformable particles in a bounded flow [44], and shear-induced diffusive flux due to hydrodynamic pair collisions between particles. Incorporating the effects of both sources of particle motion, Kumar and Graham [45] presented an idealized kinetic master equation model for a binary suspension of capsules with varying rigidity, and obtained the steady-state solution using a Monte Carlo simulation technique. They found that at low volume fractions, heterogeneous pair collisions between capsules of different components drive the segregation by rigidity difference, while at higher volume fractions, wall-induced migration and heterogeneous pair collisions have comparable contributions. The authors further revealed [42] that in a binary suspension of capsules with size contrast, the segregation behavior is mainly caused by the greater wall-induced migration velocity of the larger capsules. Similar methods have been employed to understand the dynamics and particle concentration distributions in either homogeneous suspensions or more complex binary systems [46–48]. An example is a recent work by Qi and Shaqfeh [48], who predicted the distributions of RBCs and platelets in a binary suspension subjected to pressure-driven flow.

Based on approximations that collisions only occur between closely adjacent particles and result in very small post-collisional displacements, a mechanistic theory was developed by Henríquez Rivera et al. [49] in which the master equation [45–47] was simplified into a pair of drift-diffusion equations that have been denoted as the “simplified drift-diffusion (SDD)”
model. In simple shear flow, closed-form analytical solutions for this model are attainable to describe cell-free layer formation and flow-induced segregation in binary suspensions of deformable particles. The predictions of cell-free layer thickness by this model are found [50] to agree well with the values reported in other experimental [51], numerical [42] and theoretical [47] studies. Moreover, several regimes of segregation arise, depending 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 collisional fluxes. Besides, a sharp “drainage transition”, characterized by complete depletion of one component from the bulk flow towards the walls, is also identified. This drift-diffusion model was then utilized to understand the segregation behavior in binary suspensions of spherical and ellipsoidal capsules [43], as described above, and found to well capture the key qualitative features of the shape-mediated margination and demargination. Later, this model was extended [50] to describe suspension dynamics in pressure-driven flow, which is complicated due to the nonuniform shear rate and in particular the fact that it vanishes at the centerplane. Numerical solutions predict margination regimes and a drainage transition qualitatively similar to those in the simple shear flow case.

The substantial progress by the extensive numerical and theoretical studies has not only disentangled the individual effects of the physical aspects of the particles and the suspensions in the segregation behavior, but also laid the groundwork for understanding the dynamics in more complex systems. Blood flow in SCD, as mentioned above, can considerably differ from the normal due to significant alterations in the physical properties of sickle cells compared to healthy RBCs. Deoxygenation of sickle hemoglobin (HbS) results in hydrophobic interactions among hemoglobin molecules, causing formation of long hemoglobin polymers. This polymer formation results in a variety of shapes for deoxygenated sickle cells [52], including the classic sickle shape [53]. Sickle cells also have a much smaller volume than healthy RBCs due to dehydration [54, 55]. In addition, a general increase in membrane rigidity has been determined for sickle cells compared to that of healthy RBCs [52, 56–59].

Complications associated with SCD related to RBC stiffening, which cause altered microvascular blood flow and vaso-occlusion followed by consequent tissue ischemia and infarction, have long been observed clinically. These effects only partially describe the pathophysiology of SCD, however, particularly as SCD is also known to be a vasculopathic disease in which endothelial cells that line the blood vessels are dysfunctional and inflamed. SCD vas-
culopathy bears some resemblance to cardiovascular disease, and relatedly, stroke remains a major cause of mortality in SCD. Despite such similarities, the underlying cause of chronic SCD vasculopathy remains unknown. In light of the advances in understanding the segregation behavior of flowing suspensions such as blood flow, how the abnormality in physical properties of sickle cells alters the dynamics of blood flow, and ultimately, how this alteration may be linked to the underlying mechanism for the complications in SCD, are questions to address. Cardiovascular bioengineering research has demonstrated that endothelial cells mechanotransduce biophysical cues, such as the shear forces of the hemodynamic microenvironment, into cellular biological signals [60–62]. Additionally, pathological alterations of those forces lead to activation of pro-inflammatory signals within endothelial cells and subsequent development of atherosclerotic plaques in situ that are prone to myocardial infarction and stroke [62]. While well-studied in the context of cardiovascular disease, this issue has yet to be explored in SCD. To the best of our knowledge, there exist only a limited number of computational studies regarding the behavior of sickle cells in suspensions in association with probing the mechanism for SCD complications. Examples are [63–65], which aimed to investigate the mechanism for vaso-occlusive crisis. In these studies, simulations were performed for suspensions containing sickle cells in a cylindrical tube of diameter $D = 10 \mu m$, mimicking post-capillary flow, to quantify the consequences of adhesive interactions between sickle cells and the endothelium (tube wall) during flow, and determine the effects of cell morphology and rigidity. However, in accordance with the particular focus on blood flow in post-capillaries, these studies considered suspensions with only a very small number of cells under strong confinement, so that characteristic dynamics of multicomponent suspensions such as flow-induced segregation and margination were not incorporated.

The aim of the present study is to take a first step towards understanding the dynamics of blood flow in more complex scenarios such as SCD. Using large-scale direct simulations, we investigate a number of binary suspensions containing primarily flexible healthy RBCs with a small fraction of stiff cells with different rest shapes in confined pressure-driven flow at zero Reynolds number. WBCs and platelets are not included in the suspensions due to their extremely small number fractions in blood flow. We predict that sickle cells display strong margination toward the walls due to their increased rigidity and reduced size compared to healthy RBCs. The effects of shape, size, and rigidity differences between the components on the behavior of different suspensions are studied. We also characterize the
orbital dynamics of single cells in the suspensions. Furthermore, the hydrodynamic effects of the cell suspensions on the walls, particularly the additional wall shear stress induced by the marginated cells are quantified to establish a connection with issues in SCD such as shear-induced endothelial damage.

The rest of the paper is organized as follows: in Section II we describe the models for the cells and suspension systems considered in this work, as well as the numerical methods and algorithms employed to compute the flow field and cell motion; in Section III we present detailed results and discussion on the behavior of different suspensions and the dynamics of single cells, followed by a characterization of the hydrodynamic effects of the suspensions on the walls. Concluding remarks are presented in Section IV.

II. MODEL FORMULATION

A. Model and discretization

We consider a flowing suspension of deformable fluid-filled elastic capsules in a planar slit bounded by two parallel rigid walls (FIG. 1). In the wall-normal (y) direction, no-slip boundary conditions are applied to the two rigid walls at y = 0 and y = 2H, where H is the distance from each wall to the centerplane of the slit domain. In the flow (x) and the vorticity (z) directions, periodic boundary conditions are imposed, with spatial periods Lx and Lz, respectively. The suspension is subjected to a unidirectional pressure-driven (Poiseuille) flow, and the undisturbed flow velocity field is given by

\[ u^\infty(x, y, z) = 2U_0 \frac{y}{H} \left(1 - \frac{y}{2H}\right) e_x, \]  

(1)

where \( U_0 \) is the undisturbed flow velocity at the centerplane of the channel, and \( e_x \) is the unit vector in the x direction. In the present study a constant pressure drop is imposed, which is equivalent to fixing the mean wall shear rate at \( \dot{\gamma}_w = 2U_0/H \). We take the fluids outside and within the capsules to be incompressible and Newtonian with viscosity \( \eta \) and \( \lambda \eta \), respectively, so \( \lambda \) denotes the viscosity ratio between the fluids inside and outside the capsules.

Different suspension systems comprising various types of cells are investigated in this work. A healthy RBC is modeled as a flexible capsule with a biconcave discoidal rest shape
FIG. 1: Schematic of the simulation domain.

[66, 67], with the geometry given by

\[ y = \frac{a}{2} \sqrt{1 - r^2} (C_0 + C_2 r^2 + C_4 r^4), \]  

(2)

where \( r^2 = x^2 + z^2 \leq 1 \), \( C_0 = 0.2072 \), \( C_2 = 2.0026 \), and \( C_4 = -1.1228 \); \( a \) denotes the radius of the biconcave discoid, which is \( \sim 3.8 - 4.0 \) \( \mu \)m for human RBCs. In contrast to a healthy RBC, a typical sickle cell is crescent-shaped with a characteristic length \( c \) slightly greater than \( a \) [52, 68]. In this study, a sickle cell is modeled as a stiff capsule with a curved prolate spheroidal rest shape. This shape is constructed by first polar stretching and equatorial compression of a spherical capsule with radius \( a \), which results in a slender prolate capsule with polar radius \( a_1 = 1.2a \) and equatorial radius \( a_2 = 0.25a \) (aspect ratio \( AR = a_1/a_2 = 4.8 \)). The resulting prolate capsule is then subjected to a quadratic unidirectional displacement of membrane points perpendicular to the polar axis of the capsule, which eventually generates a curved prolate capsule. Details regarding the transformation procedure for the sickle RBC model are found in [69]. For our models, the volume of a straight or curved prolate capsule is \( \sim 20\% \) that of a biconcave discoid.

Having introduced the models for the morphologies of different cells, we now describe the membrane mechanics of the capsules. In this work, the capsule membrane is modeled as an isotropic and hyperelastic surface that incorporates shear elasticity, area dilatation, and bending resistance. For an arbitrary capsule, the total energy \( E \) of the capsule membrane \( S \) is:

\[ E = \frac{K_B}{2} \int_S (2\kappa_H + c_0)^2 dS + K_B \int_S \kappa_G dS + \int_S W dS, \]  

(3)

where \( K_B \) and \( K_B \) are the bending moduli, and \( W \) is the shear strain energy density; \( \kappa_H \) and \( \kappa_G \) are the mean and Gaussian curvature of the membrane surface, respectively; \( c_0 = -2H_0 \)
is the spontaneous curvature, $H_0$ being the mean curvature of the spontaneous shape. In this equation, the first two terms represent the Canham-Helfrich bending energy [70, 71], and the third term corresponds to the shear strain energy. The behavior of the capsule membrane in response to the in-plane shear elastic force is described using a membrane model by Skalak et al. [72], in which the shear strain energy density $W$ is given by

$$W_{SK} = \frac{G}{4} \left[ (I_1^2 + 2I_1 - 2I_2) + C_a I_2^2 \right],$$

(4)

where $G$ is the in-plane shear modulus of the membrane, and $C_a$ characterizes the energy penalty for area change of the membrane. The strain invariants $I_1$ and $I_2$ are functions of the principal stretch ratios $\lambda_1$ and $\lambda_2$, defined as

$$I_1 = \lambda_1^2 + \lambda_2^2 - 2, \quad I_2 = \lambda_1^2 \lambda_2^2 - 1.$$  

(5)

Barthès-Biesel et al. [73] showed that for $C_a \gtrsim 10$, the tension of a Skalak membrane becomes nearly independent of $C_a$ under a simple uniaxial deformation, so $C_a$ is set to 10 for all capsules in our simulations. Sinha and Graham [67] have validated this model for the membrane mechanics of a healthy RBC, showing that the strain hardening behavior of the membrane predicted by this model agrees very well with the experimentally determined response of an RBC membrane to optical tweezer stretching by Mills et al. [74].

The deformability of a capsule in pressure driven flow is characterized by the dimensionless wall capillary number $Ca = \eta \dot{\gamma} l / G$, where $l$ is the characteristic length of the capsule. The bending modulus of a capsule is expressed nondimensionally by $\hat{\kappa}_B = K_B/l^2 G$, which is $\sim O(10^{-4} - 10^{-2})$ in the physiological context [75, 76]; here we set $\hat{\kappa}_B = 0.04$ for all capsules, which also prevents the cell membranes from any buckling instabilities. Taking the first variation of the total membrane energy $E$ in Eq. 3 gives the total membrane strain force density:

$$\mathbf{f^m} = \mathbf{f^b} + \mathbf{f^s},$$

(6)

where $\mathbf{f^b}$ and $\mathbf{f^s}$ are bending and shear elastic force densities, respectively. For any capsule in this work, the natural shape for shear and the spontaneous shape for bending elasticities of the capsule membrane are both chosen to be the same as the rest shape of the capsule, so that any in-plane or out-of-plane deformation would lead to an increase in the membrane energy and thus to an elastic restoring force. The capsule membrane is discretized into $N_\triangle$ piecewise flat triangular elements; in this work $N_\triangle = 320$, resulting in 162 nodes. We
have verified that increasing the number of nodes makes no difference to the cell dynamics, and this mesh resolution keeps the computational cost manageable given that very long simulation times are required for the dynamics of the suspensions to reach steady state. Based on this discretization, the calculation of the total membrane force density $f^m$ follows the work of Kumar and Graham [77] and Sinha and Graham [67] using approaches given by Charrier et al. [78] for the in-plane shear force density $f^s$ and Meyer et al. [79] for the out-of-plane bending force density $f^b$, respectively. Details regarding these calculations are found in [77] and [67].

Now we describe the suspension systems considered in this work. A base case is a homogeneous suspension of flexible biconcave discoidal capsules representing purely healthy RBCs. In our simulations Ca is always set to 1.6 for flexible biconcave discoids in all suspensions, which is in accordance with the biomechanical properties of the membrane of a healthy RBC within the physiological ranges, assuming the shear rate of blood flow in the microcirculation $\dot{\gamma}_w \sim O(10^2 - 10^3) \text{ s}^{-1}$ [80], the viscosity of plasma $\eta \sim 1.2 \text{ mPa s}$ [81], and the in-plane shear modulus of a healthy RBC membrane $G \sim 7.1 \pm 1.6 \text{ \mu N/m}$ [52].

One of the major aims of this study is to understand the effects of deformability, shape, and size contrast on the dynamics of a flowing suspension of capsules. To this end, different cases of binary suspensions are considered. The “primary” component, i.e., the component with a larger population, is always flexible biconcave discoids (healthy RBCs), and denoted as ‘p’; for all binary suspensions, $Ca_p$ is set to 1.6. The other component is stiff, termed “trace” and denoted as ‘t’. The number fractions for the capsules of these two components in all binary suspensions considered in this work are set to $X_p = 0.9$ and $X_t = 0.1$, respectively, with a number density ratio $n_p/n_t = 9$. This is particularly pertinent in SCD, as all patients have a small population (1 – 10%) of sickle cells that are permanently stiff and misshapen [82]. In the first binary suspension, the ‘t’ component is stiff biconcave discoidal capsules with the same rest shape as the flexible (healthy) ones to investigate the isolated role of rigidity difference in the dynamics of the binary suspension. In the second case, we study a binary suspension with the ‘t’ component being stiff curved prolate capsules that represent typical sickle cells, and this system serves as an idealized model for blood flow in SCD. For comparison, we also consider a suspension containing stiff straight prolate capsules with no curvature to illustrate the effect of curvature on the behavior of the suspension. In all cases of the binary suspensions, $Ca_t$ is always set to 0.4 for the ‘t’ component to control variables.
Indeed, this value is physiological given that the membrane shear modulus of a typical sickle cell is experimentally determined to be roughly four times that of a healthy RBC [52].

In this study, the volume fraction \( \phi \) of the capsules is \( \sim 0.15 \) for all suspensions. This volume fraction is consistent with the physiological hematocrit in small vessels (\( \sim 40 \, \mu m \) across) in the microcirculation [83, 84]. The domain is periodic in \( x \) and \( z \) with lengths \( L_x = L_z = 2H = 10a \), where \( a \) again is the radius of a biconcave discoid as described above. The domain height \( H = 5a \) so the confinement ratio \( C = H/a = 5 \). Furthermore, the suspending fluid and the fluid inside the capsules are always assumed to have the same viscosity, i.e., \( \lambda = 1 \) for all suspensions, to keep the computational cost manageable. This is different than the physiological viscosity ratio of \( \sim 5 \). However, our previous investigations have revealed that the dynamics of a single sickle cell [69] or prolate capsule [85] remain qualitatively unchanged as the viscosity ratio \( \lambda \) is increased from 1 to 5. Furthermore, as shown by the simulation results below (Section III A 1), the dynamics of single healthy RBCs in the suspensions assuming \( \lambda = 1 \) are consistent with the orbital behavior observed in a number of prior experimental [86–88] and numerical [89, 90] studies with \( \lambda = 5 \).

B. fluid motion

In our simulations, the particle and channel Reynolds numbers, defined as \( \text{Re}_p = \rho \dot{\gamma} w^2 / \eta \) and \( \text{Re}_c = \rho \dot{\gamma} w H^2 / \eta \), respectively, both have magnitude of \( \sim O(10^{-3} - 10^{-2}) \) based on the physiological range of the parameter values for blood flow in the microcirculation as noted in Section II A. The Reynolds numbers are assumed to be sufficiently small so that the fluid motion is governed by the Stokes equation. To determine the velocity field at each time instant, we use an accelerated boundary integral method of Kumar and Graham [77] for simulations of capsule suspensions. The fluid velocity \( \mathbf{u} \) at any point \( \mathbf{x}_0 \) in the simulation domain can be written as:

\[
    u_j(\mathbf{x}_0) = u_j^\infty(\mathbf{x}_0) + \sum_{m=1}^{N_p} \int_{S_m} q_i(\mathbf{x}) G_{ji}(\mathbf{x}_0, \mathbf{x}) dS(\mathbf{x}), \tag{7}
\]

where the single layer density \( q(\mathbf{x}_0) \) satisfies

\[
    q_j(\mathbf{x}_0) + \frac{\lambda - 1}{4\pi(\lambda + 1)} \delta_k(\mathbf{x}_0) \sum_{m=1}^{N_p} \int_{S_m} q_i(\mathbf{x}) T_{jik}(\mathbf{x}_0, \mathbf{x}) dS(\mathbf{x}) = -\frac{1}{4\pi\mu} \left( \frac{\Delta f_j(\mathbf{x}_0)}{\lambda + 1} + \frac{\lambda - 1}{\lambda + 1} f_j^\infty(\mathbf{x}_0) \right), \tag{8}
\]
Here $\mathbf{u}^\infty(\mathbf{x}_0)$ is the undisturbed fluid velocity at a given point $\mathbf{x}_0$, $S^m$ denotes the surface of capsule $m$; $\mathbf{f}^\infty(\mathbf{x}_0)$ is the traction at $\mathbf{x}_0$ due to the stress generated in the fluid corresponding to the undisturbed flow $\mathbf{u}^\infty(\mathbf{x}_0)$; $\Delta \mathbf{f}(\mathbf{x}_0)$ is the hydrodynamic traction jump across the membrane interface, which relates to the total membrane force density by $\Delta \mathbf{f}(\mathbf{x}_0) = -\mathbf{f}^m$ assuming the membrane equilibrium condition; $\mathbf{G}$ and $\mathbf{T}$ are the Green's function and its associated stress tensor.

The acceleration in this implementation is achieved by the use of the General Geometry Ewald-like Method (GGEM) by Hernandez-Ortiz et al. [91]. The key idea is to decompose the overall problem into a local and a global problem, specifically by splitting the Green's function for the Stokes equation into a singular but exponentially-decaying (short-ranged) part and a smooth but long-ranged part. Specifically, the solution associated with the local problem is obtained assuming free-space boundary conditions, i.e., it is independent of the geometry of interest. An important feature of the local solution is that it is short-ranged and therefore can be neglected beyond a length scale $\sim \alpha^{-1}$ from the pole of the local density. In this work, a cutoff length is taken as $r_{\text{cut}} = 4/\alpha$ with good accuracy, and a cell-linked algorithm [92] is applied to generate the near-neighbor list required for the computation of the local solution. The solution associated with the global problem, on the other hand, is nonsingular and ensures that the boundary conditions for the overall problem are satisfied. In this work, we compute the global solution by employing the discrete Fourier series approximation in the periodic $x$ (flow) and $z$ (vorticity) directions and the discrete Chebyshev polynomial approximation in the $y$ (wall-normal) direction. Note that in a traditional Ewald-sum-based method, the domain is triply periodic and a discrete Fourier representation is used in all three directions. In this approach, the pressure drop associated with the flow field induced by the capsules is always zero over the spatial period of the simulation domain, which ensures that the pressure drop obtained from this boundary integral implementation for a flowing suspension always equals the pressure drop in the flow in the absence of the capsules. In other words, these simulations are performed at constant pressure drop. The overall solution for the velocity or stress field is the sum of the local and global solutions. Based on extensive numerical tests [77], we set $\alpha h = 0.5$ to guarantee the convergence of the GGEM solution, where $h$ is the characteristic mesh spacing in the global problem. Once the overall solution is obtained on the mesh points, the velocity and stress at the discrete nodes on the capsule membrane are determined by 4th-order Lagrange
interpolation. For a flowing suspension in a slit geometry as considered in the present study, the computational cost of the algorithm scales as $O(N\log N)$, where $N$ is proportional to the product of the total number of capsules $N_p$ and the number of triangular elements $N_\Delta$ upon surface discretization. Details of the numerical method and algorithm are found in [77].

Once the flow field is determined, the positions of the element nodes on the discretized capsule membrane are advanced in time using the second-order explicit Adams-Bashforth method with adaptive time step $\Delta t = 0.02Ca_d$, where $d$ is the minimum node-to-node distance. Time is nondimensionalized with the wall shear rate $\dot{\gamma}_w$, and in this work, $t$ always represent dimensionless time.

III. RESULTS AND DISCUSSION

In this section, we first present and discuss simulation results for the dynamics of different suspension systems as described above (Section III A). Specifically, we focus on understanding the flow-induced segregation behavior as well as determining the orbital dynamics of single cells, especially the stiff cells, in each suspension. Substantial margination of the stiff cells is observed in all binary suspensions. Following these results, we further characterize the hydrodynamic effects of the suspensions on the walls, particularly the additional wall shear stress induced by the marginated stiff cells (Section III B). We reveal that compared to the small fluctuations in wall shear stress observed in the case of a homogeneous suspension of healthy RBCs, the marginated cells in the binary suspensions induce intermittent local wall shear stress peaks.

A. Cross-stream distribution and dynamics of cells in different suspensions

1. Homogeneous suspension of healthy (flexible) RBCs

We first consider the base case with a homogeneous suspension of flexible biconcave discoidal capsules representing purely healthy RBCs. Again, the capillary number of healthy RBCs is $Ca = 1.6$. Figure 2(a) shows a simulation snapshot for this suspension at steady state. Two periods of the simulation domain are shown in both $x$ and $z$ directions in this snapshot as well as all future snapshots for other suspensions. One observation is that RBCs are depleted in the near-wall regions. To obtain the cross-stream distribution for the cells
FIG. 2: (a) Simulation snapshot for a homogeneous suspension of flexible biconcave discoidal capsules (healthy RBCs) with Ca = 1.6. (b) Wall-normal number density profile $\hat{n}$ for healthy RBCs in a homogeneous suspension at steady state. The two positions at $y/a = 0$ and $y/a = 5$ correspond to the wall and centerplane of the channel, respectively. The error bars represent estimated error using the “blocking” method [93] in this profile and future number density profiles.

of component $\alpha$ in a general suspension, we compute the normalized number density profile of the cells based on their wall-normal center-of-mass positions, given by $\hat{n}_\alpha(y) = n_\alpha(y)/n_\alpha^0$, where $n_\alpha^0$ is the mean number density of the cells of component $\alpha$ in the suspension; for a uniform distribution, $\hat{n}_\alpha(y) = 1$ at all $y$ positions. Figure 2(b) shows the time-averaged cross-stream distribution profile for healthy RBCs in a homogeneous suspension. A cell-free
layer is evidently observed next to the wall \((y/a = 0)\), with a thickness of \(\sim 1\) radius of an RBC. The formation of this layer is attributed to the hydrodynamic interactions between the cells and the wall that lead to migration of the cells away from the wall towards the center of the channel \([44, 94]\). In addition, the two substantial peaks at \(y/a = 5\) and \(y/a \approx 1.5\) indicate that the healthy RBCs accumulate both around the centerplane of the channel and near the wall right beyond the cell-free layer. The near-wall peak is a consequence of the competition between the hydrodynamic migration away from the wall and the diffusive motion due to the interparticle collisions in a confined suspension. This peak has also been observed in simulations for suspensions of deformable spherical and ellipsoidal capsules \([42, 43]\). The peak at the centerplane results from the parabolic profile of the undisturbed velocity field in pressure-driven flow, which has also been observed in prior experiments \([95]\), simulations \([36, 96–98]\), and theoretical predictions \([50, 95]\) for suspensions of RBCs with varying volume fraction and cell deformability.

Furthermore, we determine that in the near-wall region where the local shear rate is higher, healthy RBCs approximate a rolling orbital motion, as observed in the simulation snapshot (FIG. 2(a)). Similar to our observation, this rolling orbit assumed by an RBC at high shear rate, or a transition of the cell dynamics towards the rolling orbit upon increasing shear rate, has been revealed in both experimental \([86–88]\) and numerical \([89, 90, 99]\) studies for single RBCs under physiological conditions in which the viscosity ratio between the fluids inside and outside the cell is typically \(\lambda \approx 5\), although the viscosity ratio is set to \(\lambda = 1\) in this work, again, to make the computational cost manageable. We also note, though, that the spontaneous shape has a nontrivial effect on the dynamics of single RBCs. For example, the near-wall healthy RBCs are observed to reorient from a rolling orbit into a tank-treading configuration when their spontaneous shape is changed from a biconcave discoid to an oblate spheroid. This observation is consistent with the findings in a prior numerical investigation by Sinha and Graham \([67]\). In the near-centerplane region where the local shear rate is lower, on the other hand, the cell orientations exhibit relatively larger inhomogeneity, which is likely caused by the high number density of cells near the centerplane coupled with the interparticle interactions. We have also verified that for the binary suspensions that will be presented next, the segregation behavior and dynamics of stiff cells are not affected as the orbital dynamics of healthy (flexible) RBCs change with the spontaneous shape.
FIG. 3: Time evolution of the parameter $s = \langle (y_{cm} - H)^2 \rangle^{1/2} / a$ for flexible (red) and stiff (blue) RBCs in a binary suspension.

2. Binary suspension of flexible and stiff RBCs: isolated effect of rigidity difference

We now consider a binary suspension of flexible ($\text{Ca}_p = 1.6$) and stiff ($\text{Ca}_t = 0.4$) RBCs with a number density ratio $n_p/n_t = 9$. To characterize the collective dynamics of the cells of each component, we define a parameter $s = \langle (y_{cm} - H)^2 \rangle^{1/2} / a$, which measures the root-mean-square (RMS) distance of the cells from the centerplane of the channel; here $y_{cm}$ is the center-of-mass position of an arbitrary cell in the wall-normal direction. The time evolution of $s$ for the cells of each component is plotted in FIG. 3. It is obvious that though starting with comparable values for both components, $s$ decreases for flexible RBCs while increasing for stiff RBCs, before reaching a plateau (steady state) at $s_p \approx 2.2$ and $s_t \approx 3.2$, respectively, indicating a segregation behavior in the binary suspension.

Furthermore, to quantify the cell distributions, the steady-state wall-normal number density profiles $\hat{n}(y)$ are computed for both components and shown in FIG. 4. In this case where stiff RBCs are dilute, the key observation is that, in contrast to flexible RBCs that exhibit
FIG. 4: Wall-normal number density profiles $\hat{n}$ for flexible (solid red) and stiff (dotted blue) RBCs in a binary suspension at steady state.

A profile similar to that in their pure suspension, a majority of stiff RBCs are drained from the center of the channel and aggregate in the near-wall region, undergoing substantial margination. This margination and segregation behavior is also evident in snapshots from simulation for this binary suspension (FIG. 5). We notice, nevertheless, that the position of the near-wall peak in the profile of stiff RBCs is only slightly closer to the wall compared to the peak right beyond the cell-free layer in the profile of flexible RBCs, which is mainly due to the volume exclusion effect of the marginated stiff RBCs that are found to take an approximate in-plane tumbling orbit. In addition, we have verified that the existence of a small number fraction of stiff RBCs makes little difference to the orbital dynamics of flexible RBCs (not shown).

The results above demonstrate that rigidity difference by itself is sufficient to induce the segregation behavior in a binary suspension, which has also been found in simulations for a suspension of spherical capsules with inhomogeneous rigidity [42, 50]. As an application in biotechnology, rapid separation of diseased RBCs with increased stiffness (e.g. malaria-infected RBCs) has been achieved based on cell margination or lateral displacement using
FIG. 5: Simulation snapshots ((a) side view, (b) front view) for a binary suspension of flexible (red) and stiff (blue) RBCs at steady state.

microfluidics [1, 2].

3. Binary suspension of healthy and sickle RBCs: combined effect of rigidity, shape, and size differences

In this section we present simulation results for a binary suspension of healthy and sickle RBCs, which is the focus of this work. Again, healthy RBCs are modeled as flexible biconcave discoidal capsules ($Ca_p = 1.6$), while sickle cells as stiff curved prolate capsules ($Ca_t = 0.4$); the number density ratio between these two components is $n_p/n_t = 9$. In the meantime, results for the case in which sickle cells, the trace component, are replaced by stiff straight prolate capsules ($Ca_t = 0.4$ and $X_t = 0.1$) are also presented for comparison to illustrate the effect of curvature for the trace component.

The time evolution of the parameter $s = \langle (y_{cm} - H)^2 \rangle^{1/2}/a$ is shown in FIG. 6 for each component in both suspensions. Similar to the previous case with flexible and stiff RBCs, here the segregation behavior is apparently observed for both cases. It shows from direct comparison that the evolution of $s$ for healthy RBCs is not greatly affected by the curvature of the trace component. The trends of increase in $s$ display minor differences for sickle cells and prolate capsules, both reaching a plateau (steady state) at $s_t \approx 3.6$. Note that this steady-state value is greater than that for stiff RBCs ($s_t \approx 3.2$) in a binary suspension.

More details can be revealed by computing the cross-stream distribution profiles for each component in both suspensions. In FIG. 7(a), a cell-free layer with a thickness of $\sim 1a$ is observed in the wall-normal number density profiles $\hat{n}$ for healthy RBCs in both cases,
as well as the two peaks both around the centerplane of the channel and right beyond the cell-free layer. In general, the $\hat{n}$ profiles for healthy RBCs in these two suspensions are similar to those in previous cases. For sickle cells and prolate capsules, the profiles both show a high near-wall peak inside the cell-free layer (FIG. 7(b)), which suggests that the trace component in both cases is largely drained from the bulk of the suspension, displaying strong margination as seen in the simulation snapshots in FIG. 8. No substantial differences are observed in the $\hat{n}$ profiles for both cases. Besides, we have also found that the $\hat{n}$ profiles remain nearly unchanged as the spatial period in the $x$ direction of the simulation domain is doubled from $L_x = 10a$ to $L_x = 20a$ (not shown).

It is noteworthy that the near-wall peaks in the $\hat{n}$ profiles for sickle cells and prolate capsules (FIG. 7(b)) are both of greater magnitude and closer to the wall compared to that in the profile for stiff RBCs (FIG. 4). To explain this, we first characterize the orbital dynamics of sickle cells and prolate capsules in the respective suspension. Here the cells
FIG. 7: Wall-normal number density profiles $\hat{n}$ for (a) healthy RBCs in suspension with sickle cells (solid red) and prolate capsules (dotted red), and (b) for sickle cells (solid blue) and prolate capsules (dotted blue) in suspension with healthy RBCs, respectively.

(capsules) are classified into two groups based on their wall-normal center-of-mass positions $y_{cm}$: one for the cells inside the cell-free layer, and the other for those in the bulk of the suspension. The instantaneous orientation of a single sickle cell or prolate capsule in flow is given by a unit end-to-end vector $\mathbf{p}$ that connects the two tips of the cell (capsule). We then define a tensor $\mathbf{S}$ to quantify the ensemble-averaged orientation of the cells in each group, given as

$$\mathbf{S} = \langle \mathbf{pp} \rangle = \begin{pmatrix}
\langle p_x p_x \rangle & \langle p_x p_y \rangle & \langle p_x p_z \rangle \\
\langle p_y p_x \rangle & \langle p_y p_y \rangle & \langle p_y p_z \rangle \\
\langle p_z p_x \rangle & \langle p_z p_y \rangle & \langle p_z p_z \rangle
\end{pmatrix}. \quad (9)$$

In particular, we care about the magnitude of the components $\langle p_x p_x \rangle$, $\langle p_x p_y \rangle$, $\langle p_y p_y \rangle$, and $\langle p_z p_z \rangle$, assuming that the values for the other components are essentially zero given the equal probability of $p_z$ being positive or negative for an arbitrary cell. The steady-state magnitude of each of these components is presented using a bar graph in Figure 9 for sickle cells and prolate capsules inside the cell-free layer and in the bulk of the suspension, respectively. We observe that for the cells inside the cell-free layer, the component $\langle p_z p_z \rangle$ dominates with the magnitude close to 1, while the other components are vanishingly small.
FIG. 8: Simulation snapshots (left: side view; right: front view) for binary suspensions of healthy RBCs with sickle cells (a,b) and prolate capsules (c,d), respectively, at steady state.

This suggests that the margiinated sickle cells and prolate capsules tend to approximate a log-rolling orbital motion inside the cell-free layer, with the end-to-end vector $\mathbf{p}$ nearly aligned with the $z$ axis, which is in agreement with the observations from the simulation snapshots in FIG. 8. Compared to the margiinated stiff RBCs that approximate a tumbling motion, the near-wall sickle cells or prolate capsules are able to approach closer to the walls owing to a minimal volume exclusion effect because of their near-rolling orbits, smaller volume, and slenderness in shape, which leads to a near-wall peak in the $\hat{n}$ profile that is both greater in magnitude and closer to the wall.

For sickle cells and prolate capsules in the bulk of the suspension, in contrast, $\langle p_x p_x \rangle$ dominates, with $\langle p_y p_y \rangle$ and $\langle p_z p_z \rangle$ being smaller but non-zero, indicating that the cells in the bulk generally take an averaged kayaking orbit with a small angle off the shear ($x$-$y$) plane, consistent with our previous findings on the dynamics of a single sickle cell in unbounded simple shear flow [69]. During the evolution of the suspension dynamics, this orbit becomes favorable for these slender cells with a high aspect ratio, given limited space between cells (majorly healthy RBCs) in suspension. The dynamics of healthy RBCs, nevertheless, do not show much difference compared to previous cases.
FIG. 9: Magnitude of the $x-x$, $x-y$, $y-y$, and $z-z$ components of the tensor $S = \langle pp \rangle$ for sickle cells and prolate capsules inside the cell-free layer (labelled “CFL”) and in the bulk of the suspension (labelled “bulk”), respectively.

**B. Effect of marginated cells on the walls in binary suspensions**

Having determined the cross-stream distribution and cell dynamics in different suspensions, in this section we characterize the hydrodynamic effects of the suspensions on the walls, and compare the results for the binary suspensions with the case containing purely healthy RBCs to illustrate the impact of the marginated stiff cells in the binary suspensions. Particularly, we compute the shear stress at the walls associated with different suspensions.

For an undisturbed planar pressure-driven flow in the absence of the capsules confined by two walls at $y = 0$ and $y = 2H$, the mean wall shear stress is given by $\tau_w = 2\eta U_0 / H$. In the case of a flowing suspension of capsules, however, additional wall shear stress $\hat{\tau}_w$ can be induced by the presence of the capsules. In this work $\hat{\tau}_w$ is computed numerically using an accelerated boundary integral method [77], as introduced in Section II B. Figure 10 shows
examples of the spatial distribution of $\hat{\tau}_w$ on the bottom wall ($y = 0$) induced by different suspensions at steady state. Note that this quantity and all the wall shear stress quantities reported below have been normalized by the undisturbed wall shear stress $\tau_w$ unless noted otherwise. It is clear that except for the case with purely healthy RBCs in which barely any fluctuations in wall shear stress are observed, the binary suspensions all induce local shear stress peaks on the wall, which is certainly accredited to the marginated cells in each suspension.

FIG. 10: Examples of the spatial distribution of the additional wall shear stress $\hat{\tau}_w$ on the bottom wall ($y = 0$) induced by a homogeneous suspension of healthy RBCs (a) and binary suspensions of healthy (flexible) RBCs with stiff RBCs (b), sickle cells (c), and prolate capsules (d), respectively, at steady state.
FIG. 11: Time evolution of the additional wall shear stress $\hat{\tau}_w$ at a fixed wall position $(x, y, z) = (0.74a, 0, 1.67a)$ for the cases of a homogeneous suspension of healthy RBCs (a) and binary suspensions of healthy (flexible) RBCs with stiff RBCs (b), sickle cells (c), and prolate capsules (d), respectively, at steady state.

In general, an arbitrary wall position can experience dramatic fluctuations in $\hat{\tau}_w$ due to the dynamical motion of the near-wall cells in a flowing suspension. Examples are shown in FIG. 11 for the evolution of $\hat{\tau}_w$ experienced at a fixed wall position $(x, y, z) = (0.74a, 0, 1.67a)$ for different suspensions at steady state. A number of peaks with large magnitude are observed in each case of the binary suspensions, suggesting that this wall position experiences high additional shear stress intermittently. We now detailedly characterize the hydrodynamic effect of each suspension on the walls. First, we compute the RMS of the additional wall shear stress $\hat{\tau}_w$ for each wall position, and the results for the spatially-averaged RMS wall shear stress, $\langle (\hat{\tau}_w)_{RMS} \rangle$, are plotted in FIG. 12(a) for different suspensions. It is ob-
served that all three types of cells as the trace component, i.e., stiff RBCs, sickle cells, and prolate capsules, cause an increase (of approximately two folds) in the averaged RMS wall shear stress in the case of the corresponding binary suspension compared to the case with purely healthy RBCs. The magnitude of the increase is the greatest for the case with prolate capsules, though the differences are small among the binary cases. In addition, the spatially-averaged maximum additional wall shear stress, $\langle \hat{\tau}_w \rangle_{\text{max}}$, is also computed for different cases (FIG. 12(b)). Again, no substantial differences are observed among the binary cases, although the magnitude of this quantity is slightly greater for the case with sickle cells than with stiff RBCs or prolate capsules.

However, despite the general indications, neither the RMS nor the maximum is able to reveal the influence of the marginated cells in a binary suspension on the walls in detail. Indeed, it is mainly these near-wall cells that arouse the intermittent peaks in the additional wall shear stress, as shown in the examples in FIG. 11. To this end, we now compute three quantities for each of the suspensions: the ensemble-averaged magnitude, duration, and frequency of the peaks in the additional wall shear stress. To do this, we consider only those wall shear stress peaks in each case that are greater than the expected maximum value for the healthy case, and the associated quantities are denoted using the subscript “$pk$”. The

FIG. 12: The spatially-averaged RMS $\langle \hat{\tau}_w \rangle_{\text{RMS}}$ (a) and maximum $\langle \hat{\tau}_w \rangle_{\text{max}}$ (b) of the additional wall shear stress for different suspensions at steady state. For each quantity, the error bars represent standard error.
FIG. 13: The ensemble-averaged magnitude $\langle (\hat{\tau}_w)_{pk} \rangle$ (a), duration $\langle \Delta t_{pk} \rangle$ (b), and frequency $\langle f_{pk} \rangle$ (c), respectively, of the wall shear stress peaks experienced by all wall positions for different suspensions at steady state, and the product $A_{pk}$ of these three quantities (d). The error bars in (a), (b), and (c) represent standard error for each quantity.

ensemble-averaged magnitude, duration, and frequency of these peaks are then computed, which gives $\langle (\hat{\tau}_w)_{pk} \rangle$, $\langle \Delta t_{pk} \rangle$, and $\langle f_{pk} \rangle$, respectively. Note that an arbitrary peak in $\hat{\tau}_w$, as defined above, is always characterized by a spike and two dips right before and after it (FIG. 11). Accordingly, the duration of a peak is defined as the time difference between the onset of the dip before the spike and the end of the one after. Finally, we take the product
As defined, each suspension exerts a substantial effect on the wall shear stress. The results are summarized in FIG. 13. It is obvious that compared to the case with purely healthy RBCs where $A_{pk}$ is vanishingly small, all three binary suspensions exert a substantial overall effect on the wall shear stress (FIG. 13(d)). The averaged magnitude and duration of the wall shear stress peaks, $\langle \hat{\tau}_w \rangle_{pk}$ and $\langle \Delta t_{pk} \rangle$, show minor differences among the cases of binary suspensions, although both are slightly greater for the case with stiff RBCs than with sickle cells or prolate capsules. Significant differences, however, are observed in the averaged frequency of the wall shear stress peaks, with $\langle f_{pk} \rangle$ being the greatest when the trace component is prolate capsules. As a result, the product $A_{pk}$ also yields the greatest value for the case with prolate capsules, while being slightly greater for the case with sickle cells than with stiff RBCs.

The differences in $\langle f_{pk} \rangle$ among the binary suspension cases, as shown in FIG. 13(c), may be explained by the orbital dynamics of the marginated cells. As determined in Section III A 2, marginated stiff RBCs tend to approximate an in-plane tumbling orbit. Theoretically, substantial additional wall shear stress is induced when the cell is oriented perpendicular to the wall with a minimal distance between the edge of the cell and the wall. However, during the periodic orbital motion of the cell, this orientation is transient before the cell is quickly flipped by the shear effect of the flow and spend longer time at the edge of the cell-free layer becoming parallel with the wall, which conversely leads to negligible additional wall shear stress and, as a consequence, the lowest frequency of the wall shear stress peaks among the binary suspension cases. The difference in $\langle f_{pk} \rangle$ between the cases with sickle cells and prolate capsules, on the other hand, is majorly an effect of curvature: the steady rolling motion of marginated prolate capsules inside the cell-free layer, with negligible fluctuations in the wall-normal position of the cell body compared to the larger fluctuations for near-wall rolling sickle cells caused by curvature, ensures that at any wall position, a peak in wall shear stress is induced whenever a prolate capsule goes by.
IV. CONCLUSION

In this study, we investigated different flowing suspensions of deformable capsules subjected to pressure-driven flow in a planar slit using boundary integral simulations, assuming that the flow is in the Stokes regime. A base case is a homogeneous suspension of flexible biconcave discoidal capsules representing purely healthy RBCs. Additionally, three binary suspensions are studied. In one case, the two components in the suspension differ only in membrane rigidity with the same biconcave discoidal rest shape to illustrate the isolated effect of rigidity contrast. The essential case of this study, indeed, is a binary suspension of healthy RBCs and sickle cells, which describes the microvascular blood flow in sickle cell disease (SCD). The sickle cells are modeled as stiff curved prolate capsules, displaying substantial contrasts with healthy RBCs in rigidity, shape, and size. Another binary suspension containing healthy RBCs and stiff straight prolate capsules is also considered for direct comparison with the case with sickle cells to show the effect of curvature of the stiff component. In each binary suspension, the stiff component is dilute, taking up 10% of the total number of capsules.

The mechanics of the capsule membranes are described using a model incorporating both shear and bending elasticities. The parameter regime is mostly based on the range of experimentally determined values for RBCs and blood flow in the microcirculation, except that the suspending fluid and the fluids enclosed by all capsules are assumed to have the same viscosity, i.e., the viscosity ratio $\lambda = 1$, for the purpose of keeping the computational cost manageable for the simulations.

We first characterized the cross-stream distributions and orbital dynamics of different types of capsules in each suspension (Section III A), and revealed a number of key features.

(i) In the homogeneous suspension of healthy (flexible) RBCs, a cell-free layer is formed next to the channel walls. Healthy RBCs accumulate both around the centerplane of the channel and near the wall right beyond the cell-free layer, showing two corresponding peaks in the number density distribution profile. In the near-wall region where local shear rate is high, healthy RBCs approximate a rolling orbit with the major axis nearly aligned with the vorticity ($z$) direction, which is in agreement with the observations in prior studies [86–90] for the dynamics of single RBCs at high shear rate with a more physiological viscosity ratio $\lambda = 5$. No qualitative changes are observed for the distribution profile and dynamics of
healthy (flexible) RBCs in binary suspensions.

(ii) In the binary suspension of flexible and stiff RBCs with the same rest shape but large contrast in membrane rigidity, the dilute stiff RBCs are largely drained from the center of the channel and display substantial margination towards the walls, showing a high near-wall peak in the number density profile. This suggests that rigidity contrast by itself is sufficient to induce the segregation behavior in a binary suspension. The marginated stiff RBCs assume an approximate in-plane tumbling orbit.

(iii) In the binary suspension of healthy RBCs and sickle cells, the dilute stiff sickle cells are almost completely drained from the bulk of the suspension, and strongly aggregate close to the walls inside the cell-free layer. The near-wall peak in the number density profile for sickle cells is both greater in magnitude and closer to the walls than that for stiff RBCs, mainly attributed to the much smaller volume and slenderness in shape of sickle cells. The marginated sickle cells tend to roll in flow with the major axis aligned with the vorticity \( z \) direction. No substantial differences are observed for the number density profile and orbital dynamics of dilute stiff straight prolate capsules in the binary suspension compared to those in the case with sickle cells, indicating that curvature plays a minor role.

Having revealed the segregation behavior and margination of the dilute stiff component in different binary suspensions, we also quantified the physical effect of each suspension on the walls (Section III B). Particularly, the additional wall shear stress, i.e., the deviation from the undisturbed wall shear stress induced by the presence of capsules, was computed for each suspension. We found that compared to the small fluctuations in wall shear stress for the case with purely healthy RBCs, large local peaks in wall shear stress are induced by all binary suspensions, which is believed to be a hydrodynamic effect of the marginated cells on the walls. Detailed quantification showed that the shape and size of the stiff component have a secondary role, even though for the parameter space considered in this study, straight prolate capsules seem to have a greater overall effect on the wall shear stress than do stiff RBCs and sickle cells, which may be accredited to the steady near-wall rolling motion with little fluctuations in the wall-normal position and the slenderness in shape with no curvature.

Overall, all these results represent an effort to gain an improved understanding of the behavior of flowing suspensions of cells or capsules in complex scenarios, particularly the blood flow in SCD which is the focus of the current study. More importantly, this work is of essential interest from the medical point of view in that the results may help explain
the mechanism for complications associated with SCD, such as endothelial inflammation, that are still poorly understood, from a purely physical (non-chemical) perspective. For example, in SCD, endothelial cells in the microvasculature may experience intermittent elevation and disturbance in shear stress induced by sickle cells in the vicinity, as observed in our simulations, which may eventually lead to endothelial damage and inflammation. A major limitation of this work, however, is a lack of heterogeneity in cell properties, given that physiologically sickle cells vary considerably in cell morphology and membrane stiffness, which should be addressed in future studies. Other parameters of the suspensions, such as the total volume fraction and the number fraction of each component in a binary system, can also be altered in extended studies to investigate the effects of these parameters on the suspension dynamics. Furthermore, it has been observed in experiments [100] that the flow of a suspension of sickle cells around an acute corner of a triangular pillar or a bifurcation leads to an enhanced deposition and aggregation of cells. Therefore, complex geometries of the flow domain other than a planar slit should be considered in future simulations. Finally, studies are needed to elucidate if margination of stiffened RBCs in SCD in the microcirculation is adversely altered, and if so, how this process may contribute to SCD vasculopathy. Such work could directly lead to a new paradigm of biophysical therapeutic strategies directed towards mitigating the potential aberrant RBC margination effects in SCD.
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