## Soft Matter

## PAPER

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## 1 Introduction

Among all the cells, an erythrocyte possesses one of the simplest and best characterized molecular structures. For this reason it often serves as a model system for studying cellular mechanical responses.<sup>1</sup> Without a nucleus, a mature RBC contains a cytosol enclosed within a highly flexible cell membrane. The membrane features a composite structure including a fluid-like lipid bilayer on the outside strengthened by a cytoskeleton from the inside. Unlike other cells, in the RBC membrane there is only one thin layer of cytoskeleton containing major proteins such as  $\alpha$  and  $\beta$  spectrins (Sp), ankyrin, band 3, protein 4.1, protein 4.2, and actin, as well as some minor proteins such as myosin, tropomyosin (TM), and tropomodulin (E-Tmod). Structurally, the skeleton is organized into approximately 33 000 repeating units called junctional complexes (JCs). These repeating units connect with each other through the head-tohead associations of Sp from neighboring units.<sup>2</sup> Suspension complexes (SCs) function as the connections between the protein network and the lipid bilayer.3 A SC consists mainly of band 3, ankyrin, and protein 4.2.4 Among them band 3 is a

# Deformation of the erythrocyte cytoskeleton in tank treading motions

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By coupling a fluid-structure interaction algorithm with a three-level multiscale structural model, we simulate the tank treading responses of erythrocytes (red blood cells, or RBCs) in shear flows. The focus of this study is on the transient process involving the development of the local area deformation of the protein skeleton due to the slip between the lipid bilayer and the protein skeleton. The fluid motion is depicted within the Stokes-flow framework, and is mathematically formulated with the boundary integral equations. The structural model takes into account the flexible connectivity between the lipid bilayer and the protein skeleton as well as viscoelastic responses. Under the assumption that the natural biconcave configuration of the protein skeleton is its reference state (*i.e.* the state in which the skeleton has uniform pressure and zero shear stress), we reach the following conclusions: (1) during tank treading motions it takes long relaxation time for significant skeleton area deformations to establish; (2) depending on the initial orientation of the cell with respect to the incoming flow, two responding modes have been identified. One of them is characterized by oscillations in cell geometry (breathing) and orientation (swinging). The other is a steady membrane-circulating motion; (3) the oscillatory motions (breathing and swinging) are closely related to the inequality of the dimples and the rim associated with the shape memory effect. In the first response mode the regions corresponding to the dimples in the original state circulate around the cell, creating unsteadiness in the fluid-structure interaction problem and leading to oscillatory behaviors such as breathing and swinging motions.

transmembrane protein. Another site to link the protein network to the lipid bilayer is through protein 4.1 and glycophorin C (another transmembrane protein).<sup>5,6</sup>

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In summary, the basic picture of an erythrocyte membrane is that of a protein skeleton created from JCs bound to each other *via* head-to-head associations of Sp, which is anchored to the lipid bilayer primarily at isolated pinning sites (band 3 and glycophorin C).

A key structural characteristic of the RBC membrane is the flexible connectivity between its lipid bilayer and protein skeleton, enabled by the mobilities of the transmembrane proteins within the lipid bilayer (which is similar to a fluid). Consequently, during cell motions and deformations the bilayer and the skeleton can slide against each other, allowing different local deformations of the two. One implication is that although the overall surface area of the cell is conserved owing to the huge area stiffness of the lipid bilayer, there may exist local area changes of the protein skeleton. This has been confirmed through fluorescent illustration of the skeleton-binding protein density during micropipette aspirations, in which area expansion near the cap and compression near the neck of the aspirated cell were observed.7 Subsequent numerical simulations using a coarse-grained molecular dynamics model<sup>8</sup> and a multiscale model<sup>9</sup> have quantitatively reproduced the area deformation of the skeleton measured in the experiment.

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It remains unclear whether large area deformations of the protein skeleton are allowed under dynamic conditions. The aforementioned experiment and numerical simulations were all performed within the quasi-static regime, in which there is sufficient relaxation time (up to 30 min)7 for the bilayer-skeleton sliding to occur. However, under physiological conditions red blood cells circulate around the body and undergo dynamic deformations due to the combined effect of the surrounding blood flow and boundaries (e.g. capillaries or venous sinus in the spleen).<sup>10-12</sup> There is no guarantee that there is enough relaxation time. On the other hand, large (area or shear) deformations of the skeleton may trigger unfolding of domains within a protein (e.g. spectrin), or dissociation of protein-toprotein and protein-to-lipid connections. This will lead to structural remodeling, phase transition, and malfunction. It is thus critical to study skeleton deformations under dynamic conditions. Specifically, we are interested in finding whether significant local area deformations of the cytoskeleton are possible under these conditions.

One of the most intriguing dynamic responses of erythrocytes is tank treading motion, occurring in a sufficiently strong shear flow (under physiological conditions, strong shear flows exist inside the heart and major blood vessels). During such a response the cell shape and orientation remain almost steady, whereas its membrane circulates around the surface (if the shear rate is not large enough, the cell undergoes tumbling motion in which the whole cell rotates around its center, or even more complex transitional motions reported recently<sup>13,14</sup>). The deformation of the cytoskeleton during tank treading motion remains a source of controversy. It was originally suggested by Fischer<sup>15</sup> that the time scale of tank treading is too small to allow significant skeleton area deformation. In a recent simulation, however, Dodson and Dimitrakopoulos<sup>16</sup> showed that a large area change of the skeleton may indeed occur during tank treading motions. It is notable that this simulation was performed with a model in which the cell membrane is treated as a single-layer elastic material. The total surface area was conserved, whereas a local area change was permitted. The viscoelastic properties of the membrane, especially the relative motion and friction between the lipid bilayer and the skeleton, were not considered. A primary aim of the current work is to resolve this controversy of local area deformation of the RBC skeleton in tank treading motions by applying the state-of-the-art multiscale simulation technique.

In the erythrocyte membrane, viscosity comes from different sources, including: (a) the viscosity of the lipid bilayer, (b) the viscosity of the protein skeleton, and (c) the viscous connectivity between the two enabled by the mobility of the transmembrane proteins within the lipid bilayer. These effects play pivotal roles in determining the dynamic responses of the cell.

We hereby present a computational study concentrating on the local area deformations of the erythrocyte skeleton in tank treading motions. Our model combines a fluid dynamics model based on the boundary element method (BEM) and a multiscale structural model. This fully coupled fluid–structure interaction model includes the following critical mechanisms to accurately simulate dynamics of erythrocytes: (a) the interaction between inner/outer fluids and the composite membrane, (b) the viscoelasticity of the membrane, (c) the interaction between the lipid bilayer and the protein skeleton, and (d) the detailed architecture and mechanics of the protein skeleton. A key difference between this model and the one used by Dodson and Dimitrakopoulos<sup>16</sup> is that in our model we take full account of the flexible connectivity between the lipid bilayer and the skeleton. This gives us the capability to simulate the transient processes involved in the sliding motions between the skeleton and the lipid bilayer, which is critical in predicting the dynamic process of skeleton deformations.

The development and validation of our model have been recently published.<sup>17</sup> In that work we studied tank treading motion in its initial phase and the tank treading frequencies, as demonstrations of the capability and accuracy of the model. In our current work, we will investigate the long-term evolution of skeleton deformations during tank treading motions with hundreds of tank treading cycles, which is critical to answer the question whether the local area of the skeleton is conserved after accumulated deformation.<sup>15</sup> We will also focus on the effect of initial cell orientation and the exact distribution of deformations on the cell surface as well as its impact upon dynamic behaviors of the cell. To achieve these goals, improvements in the existing numerical algorithm have been made to increase its robustness in long-term simulations as well.

The rest of the paper is organized as follows. We first present a description of the physical problem and the model, including the multiscale representation of the erythrocyte membrane as well as mathematical models of the viscoelastic behavior and the fluid–structure interactions. In the section of results, the area deformations of the protein skeleton are illustrated through the time histories of the maximum area change and its distribution over the cell. Other effects, such as swinging and breathing motions (especially the correlation between them and the deformation of the skeleton), will also be discussed. Finally, conclusions are drawn.

## 2 Numerical model

The physical model and numerical methodology utilized in this study stem from a sequence of models about erythrocyte mechanics we developed in recent years, including a force-elongation model based on thermally activated theory to model the mechanics of Sp (including its folding/unfolding reactions),18 a molecular-detailed dynamics model of JC,19 a quasi-static multiscale model of the composite membrane,9 and a viscoelastic model that accounts for fluid-structure interactions.17 These models have been tested extensively through comparisons with theoretical, experimental, and numerical results in the literature. For example, the Sp model was validated by comparing the predicted tension-elongation curves with AFM measurements by Rief et al.<sup>20</sup> (see Zhu and Asaro 2008).<sup>18</sup> The JC model was tested by comparing the orientations of the actin protofilaments with experimental observations by Bennett and Baines,21 and the predicted shear stiffness of the skeleton to various experiments.19 In addition, we tested the multiscale model by comparing the resting cell shape with theoretical predictions by Seifert et al.,22 and by comparing mechanically induced cell deformations with

results from micropipette aspirations<sup>23</sup> and optical tweezer experiments<sup>24</sup> (see Peng *et al.* 2010).<sup>9</sup> In that study, we not only tested the overall cell deformation, but also the experimentally measured skeleton deformation.<sup>7</sup> Finally, the flow-induced cell motions and deformations have been compared with experiments and other numerical simulations (*e.g.* cell motion inside a tube, as well as tumbling and tank treading motions of cells in shear flows).<sup>17</sup>

In the following we briefly review the multiscale rendition and the viscoelastic/fluid-structure interaction models. In comparison with the model described in Peng et al. 2011,<sup>17</sup> the primary change in the current work lies in the application of isoparametric quadrilateral elements in both finite element method (FEM) and the boundary element method (BEM), which improves the accuracy and stability of the model. Other improvements in the detailed numerical implementation have also been made to enable long-term simulations, e.g. the application of selective reduced integration (2-by-2 in-plane Gaussian points) in the shell elements rather than uniform reduced integration (1 in-plane Gaussian point) to eliminate the hourglass mode accumulated in long-term simulations,25 and the application of the principle of virtual work to establish the relationship between the surface traction and nodal force in the BEM by solving a linear equation.

#### 2.1 Multiscale representation

The multiscale rendition consists of three models characterized by different length scales, referred to as Level I, II, and III models. At the complete cell level (Level III) the membrane is modeled as two distinct layers of continuum shells using FEM, in which the skeleton-bilayer interactions are depicted as a slide in the lateral (*i.e.* in-plane) direction and a normal contact force. The constitutive laws of the inner layer (the protein skeleton) are obtained from a molecular-detailed model (Level II). The mechanical properties of Sp, including its domain folding/unfolding reactions, are obtained with a molecular-based thermally activated constitutive model (Level I). These three models are coupled through an information-passing multiscale algorithm, in which predictions of Level I and Level II models are employed as constitutive laws in the Level II and Level III models, respectively. For completeness, hereby we briefly summarize the key characteristics of each of these models. Details of these models are given in our previous publications.9,18,19

Level I (Sp model). Sp contains multiple folded domains, which can undergo reversible unfolding under sufficiently large mechanical loads (Fig. 1c). As observed in atomic force microscopy (AFM) experiments, the transient force–extension curve of Sp stretching displays a trademark sawtooth pattern related to unfolding of the domains.<sup>26,27</sup> This feature has been successfully reproduced using a model for both quasi-static and dynamic scenarios.<sup>18</sup> It was also found that Sp unfolding may significantly influence the bilayer-skeleton interaction in micropipette aspiration.<sup>9</sup>

Level II (molecular-detailed JC model). The force-extension relationship of the Sp obtained from the Level I model is incorporated into the molecular-detailed model of the JC which



**Fig. 1** Multiscale model of a red blood cell: Level I, Sp constitutive model; Level II, molecular-detailed junctional complex (JC) model; Level III, complete cell model. These three models are coupled through an information-passing multiscale algorithm, in which predictions of Level I and Level II models are employed as constitutive laws in the Level II and Level III models, respectively.

considers the dynamic response of the fully coupled skeletonbilayer structure (Fig. 1b). Our model of a JC is based on the three-dimensional model of a single JC unit by Sung and Vera<sup>28</sup> and the numerical model by Zhu *et al.*<sup>19</sup> The junction between the Sp and the actin protofilament and the Sp-bilayer/actinbilayer interactions are all based on the state-of-the-art understanding of the actual molecular architecture so that this model is as close to reality as possible. It thus provides a unique capability of predicting the mesoscale mechanics of single or multiple units of the cytoskeleton network, the mechanical behavior of the lipid bilayer, and the effect of their interactions.

Level III (complete-cell model). In Level III the cell is modeled as two continuous layers, the outer one representing the lipid bilayer and the inner one representing the cytoskeleton (Fig. 1a). The interaction between the inner and the outer layers is simulated by considering two issues, normal contact (normal with respect to the lipid bilayer) and lateral slide (enabled by the mobility of the skeleton-bilayer pinning points within the bilayer). Specifically, we treat such an interaction as a linear spring-softened contact in the normal direction, and a viscous friction in the tangential direction. The properties of the friction are determined by the diffusion of transmembrane proteins,<sup>29,30</sup> and contribute to the dynamic response of the composite structure. Owing to this description, the bilayer and the skeleton are allowed to have different local deformations, although the overall surface area is conserved. For general deformations, we use shell elements with constant thickness to simulate both layers. Although the constitutive properties of the lipid bilayer are taken from measurements, the properties of the skeleton are not readily available and are therefore calculated by using the Level II model. In comparison with our previous work,<sup>17</sup> here we apply selective reduced integration (2-by-2 in-plane Gaussian points) in the shell elements rather than uniform reduced integration (1 in-plane Gaussian point) to eliminate the hourglass mode accumulated in the long-term simulation.25

#### 2.2 Dynamic modeling and fluid-structure interaction

Although the quasi-static multiscale model can be used to simulate many *in vitro* experiments with low deformation rates, erythrocytes always undergo dynamic deformation *in vivo*, *e.g.* in capillary flows and shear flows. To capture the dynamic behaviors of these cells, we have extended our quasi-static multiscale model to a dynamic model by incorporating the fluid–cell interaction, membrane viscoelasticity, and cytoskeleton dynamics.

Fluid-cell interactions. The surrounding Stokes flow is modeled using BEM, which is then coupled with FEM (the Level III model) using a staggered algorithm with explicit time integration. In our previous study,17 constant boundary elements are employed, and the surface traction is obtained using FEM and transferred to the boundary elements to calculate the fluid velocity using a lumping technique. The coordinates of the membrane are then updated using explicit time integration with the same velocity as the fluid. The detailed formulation can be found in Peng et al. 2011.<sup>17</sup> In the current study, to achieve better accuracy and stability, two major improvements have been accomplished. First, we employ isoparametric bilinear quadrilateral boundary elements instead of constant boundary elements to discretize the boundary integral equation. This leads to the same element interpolation for both FEM and BEM. Second, instead of applying the lumping technique, we follow the work by Walter et al.31 and apply the principle of virtual work to establish the relationship between the surface traction and nodal force by solving a linear equation using the linear sparse solver PARDISO<sup>32,33</sup> included in the Intel Math Kernel Library (MKL).<sup>34</sup> Our numerical experiments show that the numerical stability is significantly enhanced due to these two improvements.

**Viscoelasticity.** The viscoelasticity of the bilayer-skeleton system is essential for its ability to maintain structural stability under large dynamic loads. We use the generalized Voigt–Kelvin stress–strain relationship to model both the lipid bilayer and the cytoskeleton.<sup>35</sup> The bilayer-skeleton viscous friction and hydrodynamic drag are calculated using the transmembrane protein mobility and bilayer-skeleton hydrodynamic interaction. The viscosity of the surrounding flow is included in the fluid–cell interaction.

**Cytoskeleton dynamics.** The motion of the cytoskeleton is determined through its constitutive equations, its elastic interaction with the lipid bilayer, its viscous friction with the lipid bilayer *via* the transmembrane proteins, and its hydro-dynamic interaction with the lipid bilayer *via* the cytoplasm. We calculate the viscous friction between the lipid bilayer and the cytoskeleton due to the mobilities of the anchored proteins based on the Stokes–Einstein relationship and experimentally measured the transmembrane protein diffusivity.<sup>30</sup> The skeleton velocity is obtained based on the velocity of the lipid



h <sub>s</sub>	$\nu_{\rm s}$	$p_{\mathrm{f}}$	$p_{ m u}$	$L_{\mathrm{f}}$
2 nm	$0.05 \text{ pN} \ \mu\text{m}^{-1} \ \text{s}^{-1}$	11.12 nm	0.8 nm	6.39 nm
Lu	$\Delta\Delta x^*$	$F_{1/2}$	$\mu_{ m s}$	
39 nm	12.6 nm	12 pN	$5.7 \text{ pN} \ \mu\text{m}^{-1}$	

bilayer and the relative motion between the bilayer and the skeleton calculated from the viscous friction between the lipid bilayer and the cytoskeleton. The detailed formulation can be found in Peng *et al.* 2011.<sup>17</sup>

A key parameter that determines sliding motion between the lipid bilayer and the cytoskeleton is the friction coefficient associated with the drag forces on the transmembrane proteins, *i.e.* band 3 and glycophorin C. In our previous study,<sup>17</sup> we found that for a healthy human red blood cell, the friction coefficient between the lipid bilayer and the cytoskeleton per unit area is  $c_{\rm f} = \frac{\rho}{\rho_0} 144 \text{ pN s } \mu \text{m}^{-3}$ , where  $\rho$  and  $\rho_0$  are the current and initial protein densities of the cytoskeleton.

The parameters of the bilayer and skeleton for our multiscale representation and dynamic features are listed in Table 1 and Table 2 respectively.

#### 2.3 Validation

As mentioned earlier, our numerical method has been extensively validated in our previous publications9,17 through comparisons with experimental data and existing numerical models in different length scales, such as spectrin unfolding in AFM, optical tweezers stretching, and micropipette aspiration. In Peng et al. 2011,17 besides the comparison of the tanktreading frequency with data in the literature, we also compared the inclination angle  $\psi$  and the Taylor deformation parameter  $D_{xy}$  of a spherical capsule in a shear flow with the work by Ramanujan and Pozrikidis,<sup>39</sup> and the inclination angle  $\psi$  of a tumbling biconcave red blood cell with the work by Pozrikidis.42 To further validate our numerical algorithm, in this study we examine two additional cases. First, we study the deformation of a spherical capsule in shear flows with different capillary numbers (defined as Ca  $\equiv \eta a \beta / \mu_s$ , where  $\eta$  is the external fluid viscosity, *a* is the effective radius of the cell,  $\beta$  is the shear rate,

**Table 1** Parameters of the lipid bilayer.  $h_b$ : bilayer thickness (different from reality due to the homogeneous shell assumption);  $\mu_b$ : bilayer shear stiffness (a very small value to stabilize numerical algorithm);  $K_b$ : bilayer areal stiffness;  $s_k c_c$ : bilayer bending stiffness;  $s_k r_b$ : bilayer viscosity  $r_b$ : bilayer viscosity

$h_{ m b}$	$\mu_{ m b}$	K <sub>b</sub>	$k_{ m c}$	$\nu_{\rm b}$
2.2 nm	$10^{-3} \; \mu N \; m^{-1}$	$5\times 10^5 \ \mu N \ m^{-1}$	$2\times 10^{-19}J$	$10^{-3} \text{ pN } \mu\text{m}^{-1} \text{ s}^{-1}$



Fig. 2 Deformations of a spherical capsule in a shear flow (compared with the results by Ramanujan and Pozrikidis,<sup>39</sup> and those by Doddi and Bagchi<sup>40</sup>).

and  $\mu_s$  is the initial shear modulus of the cytoskeleton) and different viscosity contrasts ( $\Lambda \equiv \eta_i/\eta$ , where  $\eta_i$  is the internal fluid viscosity). The constitutive law reported by Ramanujan



**Fig. 3** (a) The inclination angle  $\psi$  and deformation parameter  $D_{xy}$  of an oblate capsule in a shear flow (compared with the result by Bagchi and Kalluri<sup>41</sup>). (b) The semi-major (L), minor (B) axes and the half axis length in the vorticity direction (Z) of an oblate capsule in a shear flow (compared with the result by Bagchi and Kalluri<sup>41</sup>).

and Pozrikidis<sup>39</sup> is used. In Fig. 2, we plot the predicted deformation parameter  $D_{xy}$  together with the benchmark results in the literature.<sup>39,40</sup> It is seen that our results are very close to those by Ramanujan and Pozrikidis,39 which were obtained using a boundary integral method similar to the one we use. On the other hand, there exists a nonsignificant difference between our results and those by Doddi and Bagchi<sup>40</sup> using a fronttracking method (which stems from the immersed boundary method<sup>43,44</sup>). Furthermore, we model an oblate capsule in a shear flow with a capillary number Ca = 0.05, viscosity contrast  $\Lambda = 3$ , and an aspect ratio of 0.7. We compare our results with the work by Bagchi and Kalluri<sup>41</sup> using a Skalak-type constitutive law.45 Fig. 3 shows that our results (including the inclination angle  $\psi$ , the deformation parameter  $D_{xy}$ , and the axis lengths) is in agreement with theirs in general. The quantitative difference is likely due to the differences between the boundary integral method and the front-tracking method (see Fig. 2). Such a difference can be amplified in unsteady cases with relatively large deformations.

## 3 Results

As demonstrated in Fig. 4, we consider a single erythrocyte in a linear shear flow. For the initial configuration, the cell geometry is biconcave and the skeleton has uniform pressure (hereby considered as zero) and zero shear stress (i.e. the skeleton is in the reference state).<sup>9,17</sup> In a Cartesian coordinate system (x, y)and z), the incoming flow is in the x direction and its velocity profile  $U = U(y) = \beta y$ , where  $\beta$  is the shear rate. In the following simulations, we concentrate on a shear rate of 200  $s^{-1}$ , which is considered to be the average shear rate in the human body.46 Although our model is capable of simulating cases with different interior and exterior fluid viscosities, these simulations are much more expensive than the case when these two are the same.17 In this study we concentrate on the long-term behavior (involving several hundred tank treading cycles) so that the computational time is a critical issue. A typical simulation of 80 seconds of the physical time with  $\Lambda = 1$  takes about



Fig. 4 Setup of the numerical simulations.

2-3 days to run on a 12-core Intel Xeon 2.93 GHz CPU using MPI parallelization, while it takes 20 days with  $\Lambda = 0.465$  to simulate 30 seconds of the physical time. For these reasons, in the following study we focus mostly on the case in which the exterior and interior fluids have the same viscosity. This viscosity is chosen as 0.013 pN  $\mu$ m<sup>-2</sup> s<sup>-1</sup>, which is a typical value of the effective viscosity under both experimental and in vivo conditions.<sup>47</sup> Cases in which  $\Lambda \neq 1$  will also be studied, after the behavior of the cell with  $\Lambda = 1$  is clear. The center of the cell lies on the line corresponding to zero flow velocity. The rotations of the cell around the x, y, and z axes are characterized by the roll angle  $\theta$ , the yaw angle  $\phi$ , and the pitch angle  $\psi$ , respectively. The initial orientation is thus represented by these angles at t = 0,  $\theta_0$ ,  $\phi_0$ , and  $\psi_0$ . Furthermore, to quantify the in-plane deformations of the skeleton, we define two independent parameters,  $\alpha = \lambda_1 \lambda_2$  and  $\gamma = \lambda_1 / \lambda_2$ , where  $\lambda_1$  and  $\lambda_2$  are principal in-plane stretches and  $\lambda_1 > \lambda_2$ . Here  $\alpha$  represents the area change ( $\alpha > 1$ corresponds to area expansion and  $\alpha < 1$  corresponds to area compression), and  $\gamma$  is a measure of shear deformation.

To investigate the effects of initial conditions, in this study we examine two different initial orientations: case 1 ( $\theta_0 = 0 \rightarrow \phi_0 = 0 \rightarrow \psi_0 = 45^\circ$ ) and case 2 ( $\theta_0 = 90^\circ \rightarrow \phi_0 = 45^\circ \rightarrow \psi_0 = 0$ ). Numerically, we use 1200 finite elements on both the inner and the outer layers in the Level III model (so that the overall number of finite elements is 2400). Correspondingly, the number of boundary elements is also 1200. The mesh generation is achieved by using the commercial software package ABAQUS (ABAQUS Inc., Providence, RI).

The cell is released from its initial position within the linear shear flow. After a short transient period it deforms and rotates to a new configuration. Afterwards, the cell shape and orientation may oscillate periodically around their mean values. Specifically, case 1 is characterized by periodic variations in cell shape (including compressing and stretching deformations along the direction of the incoming flow) and the pitching angle  $\psi$ . For convenience hereafter we use the same  $\theta$ ,  $\phi$ , and  $\psi$  angles defined earlier to represent the rotations of the deformed cell. For this purpose, the principal axes of inertia are obtained by calculating the moment of inertial tensor of the deformed cell. Among the three principal axes of inertia, the one with the smallest moment of inertia is the longest one, referred to as l. The angle between the x axis and the projection of l in the xy plane is the pitch angle  $\psi$ . The angle between the *x* axis and the projection of *l* in the *xz* plane is the yaw angle  $\phi$ .

The variation in cell shape and the oscillation of  $\psi$  correspond to breathing and swinging motions, respectively. These motions have been experimentally observed in experiments of red blood cells under shear flow.<sup>48,49</sup> They have also been extensively studied using computational models.<sup>1,50</sup> To quantitatively document variations of the aspect ratio during tank treading, we record the time history of the Taylor deformation parameter  $D_{xy}$  (a measure of the aspect ratio of the cell<sup>17</sup>) as well as the pitch angle  $\psi$  as shown in Fig. 5. For comparison, in the same figure we also plot the angular trajectory of a marker point A which circles around the center of the cell during the tank treading motion. The tank treading frequency predicted by the current model is around 6.7 Hz, which is consistent with our



**Fig. 5** Time histories of (a) the angular trajectory  $\psi_A(t)$  of a marker point A, (b) Taylor deformation parameter  $D_{xyy}$ , and (c) pitch angle  $\psi$ . Time t is in seconds. The initial condition is case 1.

previous predictions<sup>17</sup> and experimental measurements.<sup>47</sup> A systematic comparison of the tank treading frequency between numerical predictions and experiments is provided in our previous work for model validation.<sup>17</sup> In our current work, instead of the frequency, we will focus on the long-term evolution of skeleton deformation, the effect of initial cell orientation and the relationship between the distribution of skeleton deformation.

From Fig. 5 it is seen that the frequencies of  $D_{xy}$  and  $\psi$  variations are twice the tank treading frequency, consistent with the conclusions by Ramanujan and Pozrikidis<sup>39</sup> and Fedosov *et al.*<sup>51</sup> Case 2, on the other hand, displays periodic yawing rotations around the *y* axis during the initial stage (Fig. 6). The frequency of this motion is the same as that of the tank treading motion. This yawing motion, however, quickly decays over time and eventually disappears. A similar motion called kayaking or spinning was reported for lipid vesicles in shear flows.<sup>52</sup> The breathing and swinging responses are negligible in this case so



**Fig. 6** Time histories of the pitch angle  $\psi$ , the yaw angle  $\phi$ , and the Taylor deformation parameter  $D_{xy}$ . The initial condition is case 2.



**Fig. 7** Time evolutions of the maximum and minimum values of  $\alpha$  for (a) case 1 (wildtype cell), (b) case 2 (wildtype cell), (c) case 1 (pathological cell in spherocytosis) and (d) case 2 (pathological cell in spherocytosis). The insets in (a) and (c) provide close-up views of oscillations.

that it will eventually reach a steady state (as shown in Fig. 6) rather than the oscillatory responses (breathing and swinging) in case 1.

Although in the time histories in the left column of Fig. 5 it appears that the cell has already reached limit-cycle oscillatory responses (breathing and swinging) in case 1 after around 1 second, the evolution of skeleton area deformation over a long time shows a different scenario. In Fig. 7a and b we plot the long-term evolution of the area change characterized by the maximum value of the area deformation parameter  $\alpha_{max}$ throughout the whole cytoskeleton as well as the minimum value  $\alpha_{\min}$  for wildtype RBCs in case 1 and case 2. The maximum value of the area deformation parameter  $\alpha_{max}$  over the cell reaches its peak value twice during each tank treading cycle (there are about 458 tank treading cycles shown in Fig. 7a). For case 1,  $\alpha_{max}$  approaches 1.1 (corresponding to an area expansion of 10 percent) after 50 to 60 seconds (Fig. 7a) (to put this in perspective, we note that it has been reported that during micropipette aspirations it takes seconds for the area expansion to occur or disappear<sup>7</sup>). Meanwhile  $\alpha_{\min}$  reaches 0.9, *i.e.* 10 percent area compression. Incidentally, with the slow evolution of  $\alpha$  the oscillatory amplitudes of  $D_{xy}$  and  $\psi$  are slowly reduced (by 20% in both) and they will remain as steady values afterwards (see the right columns of Fig. 5). This is likely to be caused by the strain-induced stiffening effect of the skeleton.18

From Fig. 7a and b it is also seen that the area deformation is sensitive to the initial conditions. In case 2  $\alpha_{max}$  reaches 1.2, which is larger than its value reached in case 1 (*i.e.* 1.1). The minimum value of  $\alpha$  ( $\alpha_{min}$ ) in this case is slightly larger than its value in case 1 (*i.e.* 0.9). Indeed, not only the maximum and minimum values of  $\alpha$ , but also the distributions of it over the cell surface depend on the initial conditions. Fig. 8 demonstrates the distributions of the area deformation of the skeleton on the cell surface over half a tanking treading period (due to symmetry there is no need to show the other half) in both case 1 and case 2, and Fig. 9 shows the distributions of the shear deformation. It is clear that initial conditions play a pivotal role



**Fig. 8** Top and side views of the snapshots of the area deformation  $\alpha$  of the cell during half a tanking treading period. The small spheres mark the locations corresponding to the centers of the dimple areas in the resting state. The initial conditions are (a) case 1 and (b) case 2.



Fig. 9 Same as Fig. 8 except that the color contour shows the shear deformation  $\gamma$ .

in determining the distributions of skeleton deformations. In both cases two concentrated regions of area expansion are observed. In case 1 these two regions circulate around the cell following the tank treading motion, whereas in case 2 they stay at the two sides. Hereby an interesting finding is that the distribution of area deformation is closely related to the initial biconcave shape of the cell. Specifically, large area expansions always occur around the regions corresponding to the dimples in the original state. This, to certain extent, is reminiscent of the 'memory effect',<sup>53</sup> referring to the phenomenon that different regions (e.g. dimple and rim regions) on a cell in its natural state tend to keep their identities after deformations. In our case, this memory effect appears to be associated with the assumption that the skeleton has uniform pressure and zero shear stress in its original state so that the initial biconcave shape serves as a reference state in terms of skeleton mechanics. The shear deformation, on the other hand, varies periodically in case 1, but stay steady in case 2. Unlike the area deformation in case 1, in both case 1 and case 2 regions of large shear deformation do

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not circulate around the cell. Instead, the maximum shear deformation always occurs at the two sides of the cell, whereas the region near the plane of symmetry has small shear deformations.

Additional initial conditions (other than case 1 and case 2) have been tested, in which it is observed that the system always falls into one of the two modes similar to those in case 1 and case 2. Hereafter they are referred to as mode 1 (corresponding to case 1) and mode 2 (corresponding to case 2), respectively.

Among all the parameters, the bilayer-skeleton friction coefficient significantly affects the viscoelastic behavior of the system, especially the relaxation time of the local area deformation. Variations of this parameter may result from mutations and defects that affect the density of transmembrane proteins. For example, in spherocytes from patients with hereditary spherocytosis the density of band 3 is about  $12 \pm 4\%$  of that of healthy RBCs.<sup>54,55</sup> In addition, the diffusivity of the band 3 in human spherocytes is a little bit larger than those in normal human RBCs (although they are of the same order).<sup>30</sup> Based on these facts, we estimate the bilayer-skeleton friction coefficient in spherocytes as  $c'_{\rm f} = 10\% c_{\rm f} = \frac{\rho}{\rho_0} \cdot 14.4 \text{ pN s/}\mu\text{m}^3$ . For these cells with diminished bilayer-skeleton connectivity, the relaxation time of local area change in the skeleton can be greatly

tion time of local area change in the skeleton can be greatly reduced. According to our simulations, for these spherocytes the relaxation time is reduced by a factor of ten as shown in Fig. 7c and d. For example, in case 1 it takes only around 5 to 6 seconds to reach the steady state (these simulations are hypothetical since we did not consider the exact geometry and volume-to-surface area ratio of the spherocytes). After reaching steady states, however, the skeleton deformations are the same as those with normal bilayer-skeleton friction coefficient. Therefore, it may be relatively easy for these cells to experience significant area deformations in the cytoskeleton and there may be severe consequences. Indeed, area expansion during tank treading will further decrease the protein density and trigger structural instability so that vacancies or holes may form in the cytoskeleton and the associated bilayer will be lost, leading to decreased surface area and reduced deformability. According to recent experiments, RBCs with 18% (or more) surface area loss are completely entrapped in the spleen.<sup>56</sup>

In theoretical studies,48,57 it has been shown that the breathing and swinging motions are caused by the periodic variations of elastic energy of the membrane. From our result it is seen that these variations are associated with the shape memory effect, which causes inequality in the dimples and the rim. Indeed, our numerical tests suggest that if the shape memory effect is removed by choosing a spherical reference state for the skeleton, there will be almost no breathing or swinging motions (see Conclusions and Discussion). In mode 1, the membrane elements from the dimples circulate around the cell close to the x-y plane, causing periodic variations in the structural configuration and leading to the breathing and swinging motions. In mode 2, on the other hand, these elements stay at the two sides (far from the x-y plane) and the structural configuration of the cell remains almost steady so that there are very small breathing and swinging responses.



**Fig. 10** Time histories of the inclination angle  $\psi$  and yaw angle  $\phi$  with  $\theta_0 = 90^{\circ}$  $\rightarrow \phi_0 = 0^{\circ} \rightarrow \psi_0 = 0$ . (a)  $\Lambda = 1$ ,  $\beta = 400 \text{ s}^{-1}$ ; (b)  $\Lambda = 0.3$ ,  $\beta = 200 \text{ s}^{-1}$ .

Since there are two dimples, the frequency of breathing and swinging is twice the frequency of tank treading.

Finally, we explore the effects of viscosity contrast  $\Lambda$  and shear rate  $\beta$  upon cell responses. Their effects in mode 1 are mostly quantitative. For example, when  $\Lambda = 1$  at  $\beta = 150$  s<sup>-1</sup> the maximum area expansion is around 8%. It increases to 10% at 200 s<sup>-1</sup> and 13% at 300 s<sup>-1</sup>. These parameters, on the other hand, may trigger instability in mode 2 if  $\Lambda$  is small or  $\beta$  is large (when  $\Lambda = 1$  the threshold value of  $\beta$  for instability is around  $300 \text{ s}^{-1}$ ). Such an instability will eventually lead to the switching from mode 2 to mode 1. A typical mode switching event triggered by high shear rate is shown in Fig. 10a and 11. In this example we employ an initial orientation  $\theta_0 = 90^\circ \rightarrow \phi_0 = 0^\circ \rightarrow \psi_0 = 0$  which generates mode 2 (Fig. 11). The steady state response is quickly disturbed by a symmetry-breaking instability (see the increasing yaw motion  $\phi$  in Fig. 10a) and the system settles down at mode 1 after around 20 seconds. A similar tendency is found when the viscosity contrast is too small (see Fig. 10b for the case when  $\Lambda = 0.3$ ). We also found that the transition period from mode 2 to mode 1 increases significantly with  $\Lambda$ . For example,



**Fig. 11** Snapshots of the cell configuration with  $\theta_0 = 90^\circ \rightarrow \phi_0 = 0^\circ \rightarrow \psi_0 = 0$ ,  $\Lambda = 1$ , and  $\beta = 400 \text{ s}^{-1}$ . The contour shows the area deformation.

#### Paper

with  $\Lambda = 0.465$ , the transition period is more than 30 seconds, which takes 20 days to run the simulation, while it is about 2 seconds for  $\Lambda = 0.3$  (see Fig. 10b). For that reason, it is difficult to determine the exact threshold  $\Lambda$ . The full stability phase diagram of the two modes with respect to shear rate  $\beta$  and viscosity contrast  $\Lambda$  will be explored in the future.

## 4 Conclusions and discussion

By using a fully coupled fluid–structure interaction model, we studied the tank treading response of a red blood cell in shear flows. This model takes into account the dynamic response of the cell, including the viscoelasticity as well as the viscous friction between the lipid bilayer and the protein skeleton. A key characteristic of this model is that the cytoskeleton is allowed to slide against the bilayer, enabling local compression and expansion in it. However, due to large bilayer-skeleton friction and the large time scale of the bilayer-skeleton sliding, it takes a long transient period for significant area deformation to be accumulated. This may not be easily achievable under physiological conditions so that there is no sufficient time for area deformation to build up for wildtype RBCs *in vivo*.

Our simulations suggest that the largest area deformation always occurs around the regions that used to be the dimples in the natural biconcave state, so that the cell appears to demonstrate certain 'shape memory' behavior. Another interesting finding is that the area deformation depends on the initial condition (herein the initial orientation of the cell with respect to the incoming flow). Two different responses have been recorded. One of them (mode 1) is oscillatory (breathing and swinging). The other (mode 2) is steady. The implication is that this coupled fluid-structure system has at least two solutions. Which solution dominates the response depends on the initial setup. The oscillatory response (breathing and swinging, mode 1) is consistent with the numerical prediction by Dodson and Dimitrakopoulos.<sup>50</sup> Whereas in some experiments,<sup>47,58</sup> no pronounced oscillation (breathing and swinging) was observed, which is consistent with mode 2.

The different behaviors of mode 1 and mode 2 in terms of oscillatory motions (breathing and swinging) are consistent with the theory by Abkarian et al.48 that these oscillations are associated with the shape memory effect, which causes inequality in the dimples and the rim. After the initial elongation process the elements from the dimples and the rim have different strain-stress characteristics (as clearly shown by the difference in area deformation). In mode 1, the elements from the dimples circulate around the cell, causing periodic variations in the structural configuration and leading to the breathing and swinging motions. In mode 2, on the other hand, these elements stay at the two sides and the structural configuration of the cell remains steady so that there are very small breathing and swinging responses. It is interesting to note that vesicles may also undergo oscillatory responses (vacillatingbreathing) in shear flows, in which no skeleton deformation is involved.<sup>59</sup> The breathing-swinging (or vacillating-breathing) motions of the pure lipid vesicles are a transitional behavior between the tank treading motion and the tumbling motion.59

In the case of red blood cells, such transitional behavior is even more complex and is characterized by an extreme variation of the cell shape as illustrated in the recent work by Yazdani *et al.*<sup>13,14</sup> Our important finding is that the cell may undergo different modes of responses depending on its initial orientation. The effect of the shear rate on the stability of these modes will be explored in the future.

It is necessary to mention that the choice of the reference state of the skeleton is critical in determining cell responses. Prestress plays a significant role in cell mechanics.<sup>60</sup> Even in a red blood cell, the simplest cell in terms of internal structure, prestress may exist in both the lipid bilayer and the skeleton. The lipid bilayer is a liquid with almost zero shear stiffness. Its mechanical behavior depends mostly on its bending stiffness and curvature. The curvature corresponding to zero bending moment inside the bilayer is called the spontaneous curvature, and its effects on cell and vesicle shapes have been extensively studied.22 On the other hand, the skeleton has relatively large shear stiffness but negligible bending stiffness. The state corresponding to uniform pressure and zero shear stress inside it is its reference state. To date there is no widely accepted results about either the spontaneous curvature of the lipid bilayer or the reference state of the protein skeleton.<sup>61</sup> In our simulations, we choose the spontaneous curvature of the lipid bilayer to be zero (i.e. there is no bending moment in it when it is flat), and the biconcave shape as the reference state of the skeleton. The advantage of this assumption is that the biconcave state coincides with the lowest elastic energy stored in the membrane, providing a convenient physical explanation for the resting shape of the cell.9

To test the effect of the reference state, we start from a spherical cell (in which the skeleton is in the reference state) and deflate it until the volume-to-surface area ratio equals that of a biconcave one.9 In this case the resting shape of the cell will be a stomatocyte unless nonzero spontaneous curvatures are imposed.62 It is found that when such a cell undergoes tank treading motion in a shear flow there is almost no breathing and swinging motions. This confirms the prediction by Tsubota and Wada.63 A simple insight is that after stretching a spherical shape into an ellipsoid shape (as happens in tank treading), the final state is independent of the stretching direction. For a biconcave shape, on the other hand, the final state depends on the stretching direction. Moreover, for such a cell the stretching direction (relative to the skeleton configuration) varies during tank treading motion, causing unsteadiness. The problem of this spherical reference state is that it does not readily lead to the biconcave shape in the resting position with well-accepted mechanical properties.9,64 Additional evidence that the reference state is not a perfect sphere comes from the observation of shape memory.53 Some studies suggest that the actual reference configuration may be somewhere between a biconcave shape and a spherical shape.<sup>65-67</sup> In the future, it will be critical to determine the exact reference state of the cytoskeleton as well as its effect on the tank treading motions.

Sufficiently large skeleton deformation may trigger Sp unfolding.<sup>9,18</sup> This process not only plays a significant role in the softening behavior of the skeleton, but also affects bilayer-skeleton interactions.<sup>9</sup> In our simulations, this process is incorporated explicitly in the Level I model, and implicitly in Level II and III models through the information-passing multiscale approach. Specifically, Sp unfolding leads to dramatic softening behavior in the constitutive law used in the finite element formulation. The shear rate we consider in this study ( $200 \text{ s}^{-1}$ ) has been found to be too low to trigger unfolding. Further investigation is needed to explore the possibility of unfolding and its impact on the cell mechanics in higher shear rates. These cases are currently beyond our computational capability since they require much more computational grids for numerical stability.

One parameter that may affect the viscoelastic behavior of the cell and its tank treading dynamics is the hydrodynamic interaction between the lipid bilayer and the cytoskeleton. Based on the two-dimensional triangular spectrin network, it was calculated that such an interaction *via* the cytoplasm is much smaller than the bilayer-skeleton friction due to viscous drags of transmembrane proteins in the bilayer.<sup>17</sup> However, by using cryo-electron tomography, Nans *et al.*<sup>68</sup> recently found that the thickness of the cytoskeleton of mouse erythrocytes varied from 54 nm to 110 nm. With such a thick cytoskeleton, the hydrodynamic interaction between the lipid bilayer and the cytoskeleton *via* the cytoplasm may be significantly larger than that of an assumed two-dimensional triangular Sp network. This also needs to be investigated.

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