Multiscale modelling of erythrocytes in Stokes flow

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To quantitatively understand the correlation between the molecular structure of an erythrocyte (red blood cell, RBC) and its mechanical response, and to predict mechanically induced structural remodelling in physiological conditions, we developed a computational model by coupling a multiscale approach of RBC membranes with a boundary element method (BEM) for surrounding Stokes flows. The membrane is depicted at three levels: in the whole cell level, a finite element method (FEM) is employed to model the lipid bilayer and the cytoskeleton as two distinct layers of continuum shells. The mechanical properties of the cytoskeleton are obtained from a molecular-detailed model of the junctional complex. The spectrin, a major protein of the cytoskeleton, is simulated using a molecular-based constitutive model. The BEM model is coupled with the FEM model through a staggered coupling algorithm. Using this technique, we first simulated RBC dynamics in capillary flow and found that the protein density variation and bilayer-skeleton interaction forces are much lower than those in micropipette aspiration, and the maximum interaction force occurs at the trailing edge. Then we investigated mechanical responses of RBCs in shear flow during tumbling, tank-treading and swinging motions. The dependencies of tanktreading frequency on the blood plasma viscosity and the membrane viscosity we found match well with benchmark data. The simulation results show that during tank-treading the protein density variation is insignificant for healthy erythrocytes, but significant for cells with a smaller bilayer-skeleton friction coefficient, which may be the case in hereditary spherocytosis.

Key words: capsule/cell dynamics, microfluidics

1. Introduction

Although the mechanics of red blood cells (RBCs) has been studied for more than half a century, the structure–function relationship at the molecular level is still not completely understood. Without a nucleus, a mature RBC contains a cytosol enclosed within a highly flexible yet surprisingly strong cell membrane. This membrane is not a simple homogeneous single-layer medium. Instead, it consists of a lipid bilayer supported from the inside by the cytoskeleton, and it is essential to the structural integrity and stability of the RBC. The cytoskeleton is composed of several major

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FIGURE 1. Multiscale model of a red blood cell: (*a*) level III, complete cell model; (*b*) level II, molecular-detailed junctional complex (JC) model; and (*c*) level I, spectrin (Sp) constitutive model.

proteins: α and β spectrin (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, this skeleton is organized into approximately 33 000 repeating units called junctional complexes (JCs). As revealed by transmission electron microscopy, each JC is a small 'spoked' but edge-free hexagon, with six long $\alpha\beta$ Sp dimers radiating from a central short actin protofilament. These repeating units connect with each other through the head-to-head associations of Sp dimers from neighbouring units (Cohen, Tyler & Branton 1980). Suspension complexes (SCs) function as the primary connections between the protein network and the lipid bilayer (Bruce et al. 2003). A SC consists mainly of band 3 (a transmembrane protein), ankyrin and protein 4.2 (Bennett & Stenbuck 1979). Another site to link the protein network to the lipid bilayer involves the actin (Chang & Low 2001), where protein 4.1 is associated with the glycophorin C, another transmembrane protein with a single transmembrane domain (Reid et al. 1990). The basic molecular architecture of a JC, and its connectivity with the lipid bilayer, is shown in figure 1(b). To illustrate the structural response of RBCs, it is necessary to build comprehensive models that includes sufficient molecular details rather than simple continuum models. Knowledge about the detailed structure versus response properties is especially important in understanding the mechanics of diseased or aged cells.

During circulation an RBC sustains large dynamic deformation owing to the combined effect of the fluid loading and confinement within capillaries and slits of venous sinuses (e.g. in the spleen) (Mebius & Kraal 2004). The loading associated with such deformation may affect the structural integrity of the cell (especially for those cells with molecular defects), as manifested in structural remodelling, structural failure and cell dysfunction. The possibility of flow-induced cell damage is more pronounced within artificially created flow fields inside mechanical circulatory support

apparatus (for example within artificial blood pumps). It has been reported that flow with high shear rates and strong turbulence inside artificial heart valves can destroy these cells, causing blood haemolysis (see for example Deutsch *et al.* 2006).

RBC diseases (e.g. hereditary spherocytosis, malaria, and sickle cell disease) are often associated with defects or mutations of proteins, their inter-connectivity and their connectivity with the lipid bilayer. These molecular-level changes are usually manifested in changes of the overall mechanical properties of the cell. For example, under these conditions the cells may become stiffer, with reduced capacity to pass through narrow confined openings such as capillaries and slits inside the spleen. One of the most common diseases is hereditary spherocytosis, in which cells become spherical due to partial loss of the lipid bilayer. To explain the bilayer loss, a hypothesis based on the bilayer-skeleton detachment has been suggested (Walensky, Mohandas & Lux 2003). According to this hypothesis, the skeleton density is significantly decreased and the linkage between the lipid bilayer and the skeleton may break under mechanical loads so that the lipid bilayer will separate from the skeleton. Loss of lipid bilayer may occur in other circumstances. For example, it has been suggested that during its 120-day lifetime, the RBC may gradually lose some lipid bilayer as it passes through the spleen. Its surface-to-volume ratio will increase during this aging process, eventually leading to its 'death' inside the spleen (Waugh et al. 1992).

In addition to bilayer loss, large skeleton deformations may trigger other structural remodelling such as Sp unfolding or dissociation of the head-to-head connection between Sp dimers (Li *et al.* 2007) (which causes a phase transition of the skeleton). To understand these processes, as well as the dissociation of the skeleton from the bilayer, it is critical to quantitatively predict the mechanical loads on the inter-protein, intra-protein and protein-to-bilayer connections. In the following, we briefly review the existing studies and show that it is still difficult to predict these mechanical loads in the molecular level, especially the bilayer–skeleton interaction force, through existing modelling and experimental methods.

Owing to its important physiological function and structural simplicity, the mechanics of the RBC has been studied extensively during the past 50 years. Existing studies fall into three categories: (a) those concentrating on the macroscopic response of the complete cell, including experimental investigations using micropipettes (see for example Waugh & Evans 1979; Discher, Mohandas & Evans 1994), optical tweezers (Henon 1999; Sleep et al. 1999; Dao, Lim & Suresh 2003), optical magnetic twisting cytometry (Puig-De-Morales-Marinkovic et al. 2007), full-field laser interferometry techniques (Park et al. 2009), as well as numerical models of complete cells without considering effects of surrounding fluids (Discher, Boal & Boey 1998; Dao, Li & Suresh 2006; Li et al. 2007; Kabaso et al. 2010); (b) those focusing on the mechanical response and constitutive properties of single molecules or interconnectivity between molecules, for example that for Sp (Rief et al. 1997; Law et al. 2003) or for ankryn (Lee *et al.* 2006), by using atomic force microscopy (AFM) or molecular-dynamics (MD) simulations; (c) those focusing on fluid-structure interactions, including in vitro experiments using flow channels (Hochmuth 1973; Berk & Hochmuth 1992), microfluidic tools (see for example Fischer 2004; Abkarian, Faivre & Viallat 2007), as well as various analytical and numerical studies (see for example Pozrikidis 2003a, 2010). Since the primary goal of our research is to relate the fluid-structure interaction of RBCs in Stokes flow with detailed mechanical loading and structural deformation inside its molecular architecture, a more detailed review is given on the third category as follows.

The asymptotic theories by Barthès-Biesel (1980) and Barthès-Biesel & Rallison (1981) show the influence of interfacial elasticity on the small deformation of a capsule consisting of a thin elastic solid skin, enclosing a Newtonian incompressible liquid, and the rheology of dilute suspensions of capsules. Keller & Skalak (1982) studied the motion of a tank-treading ellipsoidal particle in a shear flow using an analytical approach. Secomb et al. (1986) applied lubrication theory to investigate the motion of axisymmetric RBCs in narrow capillaries (see also Halpern & Secomb 1992). Skotheim & Secomb (2007) obtained the complete phase diagram from tumbling to tank-treading motions for RBCs in shear flow. Following these analytical studies, various numerical models were developed to study large deformation and non-axisymmetric cases of capsules in Stokes flows, including the boundary element methods (BEMs) developed by Pozrikidis (1990), Zhou & Pozrikidis (1990), Ramanujan & Pozrikidis (1998), Pozrikidis (2001, 2003b, 2005), Lac et al. (2004), Kessler, Finken & Seifert (2004) and Zhao et al. (2010), the immersed boundary methods by Eggleton & Popel (1998), Bagchi (2007), Zhang, Johnson & Popel (2008) and Le (2010). The RBC membrane is considered as a uniform continuum media in these models, and the detailed molecular structure was not considered.

Recently multiscale models have been developed to study the static and dynamic response of RBCs (see for example Fedosov, Caswell & Karniadakis 2010; Fedosov *et al.* 2011). Omori *et al.* (2011) also compared different spring network models and continuum models of capsules in shear flow. These models, however, do not explicitly address the detailed internal connectivity of the cell (e.g. the connectivity between the protein skeleton and the lipid bilayer). Also, important molecular-level processes such as protein unfolding were not considered due to the absence of models for molecular connectivity. Controversies arise due to the lack of detailed depiction of internal connectivity. For example, it is still not clear whether the density of the cytoskeleton will change significantly during tank-treading motions. According to the model by Dodson & Dimitrakopoulos (2010), considerable areal dilatation of cytoskeleton is possible. This is in contradiction with Fischer (1992), who found that during tank treading there was not enough time for the bilayer–skeleton slip to happen.

It is clear that the quantitative prediction of detailed force distributions inside a cell (especially the force on structural connection points), is beyond the capacity of existing models. Similarly, state-of-the-art experimental techniques are not capable of resolving the distribution of mechanical loads inside a cell. Towards this end, a numerical model that includes sufficient molecular details is needed.

In this paper, we develop a RBC model with a fluid-structure interaction algorithm stemming from the approach by Walter *et al.* (2010) (see also Foessel *et al.* 2011; Walter, Salsac & Barthès-Biesel 2011) by including the bending stiffness of the lipid bilayer using the well-established continuum-based shell element method (Belytschko, Liu & Moran 2000; Hughes & Liu 1981b). More important, we also incorporate a multiscale model of the molecular structure of the RBC membrane in order to study the inter-molecular forces between the lipid bilayer and the cytoskeleton in different kinds of Stokes flow with physiological relevance. As an initial step in this direction, hereby we concentrate on a molecular-detailed model of healthy RBCs in two physiologically relevant Stokes flow conditions, the capillary flow and the shear flow. Studies on bilayer-skeleton interaction forces at the molecular level of different diseased cells, e.g. hereditary spherocytosis, malaria and sickle cell disease, will be carried out in the future.

To simulate the dynamic responses of RBCs in flow fields, we extend the quasistatic multiscale model of the RBC membrane (Peng, Asaro & Zhu 2010) to a dynamic version by including the fluid-structure interaction and the membrane viscoelasticity. First, we briefly review the quasi-static multiscale method in § 2.1. Then in § 2.2 we develop an approach to solve the fluid-cell interaction problem by coupling the FEM and the BEM. The membrane viscoelasticity model and the cytoskeleton dynamics are also included. Numerical results, including the deformation of an RBC inside a tube, and the tumbling, tank-treading and swinging motions of an RBC in shear flow, are presented. Comparisons between our multiscale model and simpler continuum models have also been carried out. Finally, conclusions are drawn.

2. Problem description and mathematical formulations

2.1. Multiscale method

The quasi-static multiscale method consists of three models characterized by different length scales. These are referred to models at levels I, II and III. At the complete cell level (level III) the membrane is modelled as two distinct layers of continuum shells using the finite element method, in which the skeleton–bilayer interactions are depicted as a slide in the lateral (i.e. in-plane) direction (caused by the mobility of the skeleton–bilayer pinning points) 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 the 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 in the following we briefly summarize key characteristics of each of these models. Details of these models are given in our previous publications (Zhu *et al.* 2007; Zhu & Asaro 2008; Peng *et al.* 2010).

Level I (Sp model)

Sp contains multiple folded domains, which can undergo reversible unfolding under sufficiently large mechanical loads (figure 1c). As observed in AFM experiments, the transient force–extension curve of Sp stretching displays a trademark sawtooth pattern related to unfolding of the domains (Rief *et al.* 1997; Law *et al.* 2003). This feature has been successfully reproduced using a model for both quasi-static and dynamic scenarios (Zhu & Asaro 2008).

Level II (molecular-detailed JC model)

The force-extension relation of the Sp obtained from the level I model is incorporated into the molecular-detailed model of the JC which considers the dynamic response of the fully coupled skeleton-bilayer structure (figure 1b). Our model of a JC is based upon the three-dimensional model of a single JC unit by Sung & Vera (2003) and the numerical model by Zhu *et al.* (2007). The junction between the Sp and the actin protofilament and the Sp-bilayer/actin-bilayer interactions are all based upon 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 behaviour of the lipid bilayer and the effect of their interactions.

Level III (complete-cell model)

In level III the cell is modelled as two continuous layers, the outer one representing the lipid bilayer and the inner one representing the cytoskeleton (figure 1a). The interaction between the inner and the outer layers is simulated by considering



FIGURE 2. Schematic of a red blood cell immersed in two fluids with different viscosities (the cytoskeleton is not drawn and the dashed line denotes the middle surface of the lipid bilayer).

two issues, normal contact 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 (normal with respect to the lipid bilayer), and a viscous friction in the tangential direction. The properties of the friction are determined by the diffusion of transmembrane proteins (see for example Kapitza *et al.* 1984; Kodippili *et al.* 2009), 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 skeleton are not readily available and are therefore calculated by using the level II model.

In this paper, both three-dimensional and axisymmetric studies are conducted. Threedimensional simulations are used for an RBC in shear flow, while axisymmetric simulations are used for RBCs in capillary flow. In the following we focus mostly on the general formulation for three-dimensional problems. Special aspects for axisymmetric problems will also be mentioned briefly. In these simulations, the reference configuration (stress-free state) for the cytoskeleton is always chosen to be its natural biconcave shape. The spontaneous curvature (Seifert, Berndl & Lipowsky 1991) of the lipid bilayer is chosen to be zero.

2.2. Fluid-cell interactions

We consider an RBC filled with an interior fluid (cytoplasma, hereafter referred to as fluid 2) and immersed in an exterior fluid (e.g. blood plasma, referred to as fluid 1) as illustrated in figure 2. Here Ω^f and Ω^b are the volumes occupied by the fluids and the bilayer, respectively. The boundary between the bilayer and the exterior fluid is $\Gamma^{fb,1}$. The boundary between the bilayer and the interior fluid is $\Gamma^{fb,2}$. For a general problem, two types of boundary conditions are specified: Dirichlet boundary Γ^{fD} and Neumann boundary Γ^{fN} . The entire computational domain is $\Omega = \Omega^b \cup \Omega^f$ and the entire boundary is $\Gamma = \Gamma^{fN} \cup \Gamma^{fD} \cup \Gamma^{fb,1} \cup \Gamma^{fb,2}$. Hereafter we use the superscript 'b' to represent the lipid bilayer, 'f' the fluid and 's' the cytoskeleton. Furthermore, we use bold uppercase symbols to represent tensors or multi-column matrices and bold lowercase symbols to represent vectors or single-column matrices.

2.2.1. Governing equations

The dynamic responses of both the lipid bilayer and the fluids are controlled by the conservation of momentum and the conservation of mass. No body force is considered here, and the inertial force is negligible in this length scale. With the updated Lagrangian description, the governing equation of lipid bilayer is

$$\nabla \cdot \boldsymbol{\Theta}^b = \mathbf{0},\tag{2.1}$$

where ∇ is the spatial gradient operator and Θ^b is the Cauchy stress tensor inside Ω^b . The constitutive equations of the lipid bilayer from which Θ^b is obtained are presented in § 2.2.3. In (2.1) and what follows, the single dot denotes the scalar product.

Within the Eulerian description, the Stokes equation and the continuity equation for interior/exterior Newtonian fluids are expressed as

$$\nabla \cdot \boldsymbol{\Theta}^{f} = -\nabla p^{f} + \eta_{l} \nabla^{2} \boldsymbol{v}^{f} = \boldsymbol{0}, \qquad (2.2)$$

$$\nabla \cdot \boldsymbol{v}^f = 0, \tag{2.3}$$

where Θ^f is the Cauchy stress tensor inside the fluids, v^f is the fluid velocity vector, p^f is the fluid pressure and η_l is the dynamic viscosity of fluid (l = 1, 2 stand) for the exterior and interior fluids, respectively). For normal *in vivo* RBCs, $\eta_1 = 1.2 \text{ cP} = 0.0012 \text{ pN} \text{ µm}^{-2} \text{ s}$ and $\eta_2 = 6 \text{ cP} = 0.006 \text{ pN} \text{ µm}^{-2} \text{ s}$ (see for example Chien 1987). $\Lambda = \eta_2/\eta_1$ is the viscosity contrast ratio.

The boundary conditions are given as

$$\boldsymbol{v}^{f} = \bar{\boldsymbol{v}}^{fD}$$
 on Γ^{fD} , (2.4)

$$\boldsymbol{t}^{f} = \boldsymbol{\bar{t}}^{fN} \quad \text{on } \Gamma^{fN}, \tag{2.5}$$

$$\boldsymbol{v}^{b} = \boldsymbol{v}^{f} \quad \text{on } \Gamma^{fb,1} \text{ and } \Gamma^{fb,2},$$
(2.6)

$$\boldsymbol{t}^{b} = \boldsymbol{t}^{f} \quad \text{on } \Gamma^{fb,1}, \tag{2.7}$$

$$\boldsymbol{t}^{b} - \boldsymbol{\tau}^{bs} = \boldsymbol{t}^{f} \quad \text{on } \Gamma^{fb,2}, \tag{2.8}$$

where $\bar{\boldsymbol{v}}^{fD}$ is the prescribed velocity vector on Γ^{fD} , and $\bar{\boldsymbol{t}}^{fN}$ is the prescribed traction vector on Γ^{fN} , $\boldsymbol{t}^b = \Theta^b \cdot \boldsymbol{n}$ and $\boldsymbol{t}^f = \Theta^f \cdot \boldsymbol{n}$ are the surface tractions of the bilayer and fluid domains (traction is defined as force per unit area on a surface), \boldsymbol{n} is the normal vector of the boundaries pointing towards fluid 1 and $\boldsymbol{\tau}^{bs}$ is the bilayer–skeleton interaction force per unit area applied on the cytoskeleton, which is presented in § 2.3.

In our approach, the lipid bilayer is modelled as a viscoelastic solid with tiny shear stiffness and large area stiffness. In reality the lipid bilayer is close to a fluid so that the current viscoelastic model of the lipid bilayer is just an approximation. To ensure the accuracy of this treatment, two characteristics are incorporated: (a) the shear stiffness of the bilayer is much smaller than that of the protein network, so that the later dominates the shear stiffness of the overall composite structure; (b) the skeleton-bilayer pinning points (the transmembrane proteins) can move inside the lipid bilayer, so that the skeleton is able to drift against the bilayer. In this approach, the shear stiffness of the lipid bilayer is not chosen to be zero due to numerical reasons. Equation (2.1), together with the constitutive relations, is solved through a finite element algorithm using shell elements (\S 2.2.2). Shell elements with absolute zero in-plane shear stiffness is not numerically stable in a Lagrangian description. Nevertheless, this simple model of the lipid bilayer delivers sufficient accuracy in the testing cases reported in this paper.

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A boundary element algorithm is adopted to solve dynamics of both the interior and the exterior fluids. The basic formulations of this method are summarized in 2.2.5.

2.2.2. Variational form of bilayer equations and finite element discretization

Let $V = V(\Omega^b)$ denote the trial function space for the displacement u^b and $W = W(\Omega^b)$ the test function space for the momentum equation (2.1). By using the principle of virtual power (Belytschko *et al.* 2000), the variational form (weak form) of (2.1) with its boundary conditions is stated as finding $u^b \in V$ such that for $\forall \delta v^b \in W$,

$$\iiint_{\Omega^b} \nabla \delta \boldsymbol{v}^b : \boldsymbol{\Theta}^b \,\mathrm{d}\Omega^b = \iint_{\Gamma^{fb,2}} \delta \boldsymbol{v}^b \cdot \boldsymbol{\tau}^{bs} \,\mathrm{d}\Gamma + \iint_{\Gamma^{fb,1} \cup \Gamma^{fb,2}} \delta \boldsymbol{v}^b \cdot \boldsymbol{t}^f \,\mathrm{d}\Gamma, \quad (2.9)$$

where $\boldsymbol{v}^b = \partial \boldsymbol{u}^b / \partial t$ is the velocity vector of the lipid bilayer.

Numerically, we employ the FEM to solve (2.9) by modelling the lipid bilayer as congregations of shell elements. For simplicity and numerical robustness, in our current study we choose the C^0 explicit Hughes–Liu elements (Hughes & Liu 1981*b*), i.e. the continuum-based shell element (Belytschko *et al.* 2000), which is based on the Reisser–Mindlin shell theory in which transverse shear deformation is allowed.

In this approach, a bi-unit cube within the (ξ, η, ζ) space is mapped to the geometry of a shell element based on the iso-parametric representation as shown in figure 3. In the physical space, the lines corresponding to constant ξ and η (i.e. the lines in ζ direction) are called *fibres*. The surfaces of constant ζ are called *laminae*. Four nodes are chosen at $\zeta = 0$, the middle lamina which is called the *reference surface*. With the bilinear in-plane interpolation, a point **x** on a shell element is expressed as

$$\boldsymbol{x}(\xi,\eta,\zeta) = \bar{\boldsymbol{x}}(\xi,\eta) + \boldsymbol{x}'(\xi,\eta,\zeta), \qquad (2.10)$$

where

$$\bar{\mathbf{x}}(\xi,\eta) = \sum_{a=1}^{4} N^{(a)}(\xi,\eta) \bar{\mathbf{x}}^{(a)}$$
(2.11)

and

$$\mathbf{x}'(\xi,\eta,\zeta) = \sum_{a=1}^{4} N^{(a)}(\xi,\eta) \mathbf{x}'^{(a)}(\zeta).$$
(2.12)

Let \bar{x} denote the position vector of a point at the reference surface and x' denote the point vector which starts from \bar{x} and points towards the fibre direction, while a = 1, 2, 3, 4 correspond to the four nodes shown in figure 3. The superscript '(a)' denotes quantities at node 'a'. For example, $\bar{x}^{(1)}$ and $x'^{(1)}$ are the position and the point vectors at node '1', respectively. The shape (interpolation) functions $N^{(a)}$ at node 'a' are given as $N^{(1)} = (1/4)(1 - \xi)(1 - \eta)$, $N^{(2)} = (1/4)(1 + \xi)(1 - \eta)$, $N^{(3)} = (1/4)(1+\xi)(1+\eta)$ and $N^{(4)} = (1/4)(1-\xi)(1+\eta)$. Uniformly reduced integration with one in-plane Gaussian point at the centre and three Gaussian points in the thickness direction is applied to integrate (2.9) (Belytschko *et al.* 2000).

By using the shell element representation, the lipid bilayer domain Ω^{b} between the surfaces $\Gamma^{fb,1}$ and $\Gamma^{fb,2}$ is represented by a single middle reference surface Γ^{fb} shown as the dashed line in figure 2. After finite element discretization (detailed formulation of this method can be found in Hughes & Liu 1981*b* and axisymmetric formulation can be found in Hughes & Liu 1981*a*), the governing (2.1) is re-expressed as



FIGURE 3. Three-dimensional iso-parametric mapping from a bi-unit cube to the physical shell element domain.

 $6N_{FE}$ (N_{FE} is the number of nodes in finite elements) algebra equations symbolically expressed as

$$f_{FE}^{b} = f_{FE}^{bs} + f_{FE}^{fb}, \qquad (2.13)$$

$$m_{FE}^b = m_{FE}^{bs} + m_{FE}^{tb},$$
 (2.14)

where f_{FE}^{b} is the global nodal vector of internal force related to material constitutive equations of the lipid bilayer, f_{FE}^{bs} is the global nodal vector of external force from the bilayer–skeleton interaction and f_{FE}^{fb} is the global nodal vector of fluid-bilayer interaction force on the surface Γ^{fb} , while m_{FE}^{b} is the global nodal vector of internal moment of the lipid bilayer, m_{FE}^{bs} is the global nodal vector of external moment from the bilayer–skeleton interaction and m_{FE}^{fb} is the global nodal vector of the fluid–bilayer interaction moment on the surface Γ^{fb} . All of these vectors have the dimension of $3N_{FE}$. The exact forms of these vectors are found in Hughes & Liu (1981b).

2.2.3. Constitutive laws

The viscoelasticity of the bilayer–skeleton system is essential for its ability to maintain structural stability under large dynamic loads. The viscosity stems from the following sources: (a) the viscosity of the lipid bilayer, (b) the viscosity of the cytoskeleton, (c) the skeleton–bilayer viscous friction due to the mobility of the transmembrane proteins (band 3 and glycophorin C) within the lipid bilayer and the bilayer–skeleton hydrodynamic drags and (d) the viscosity of the surrounding flow. Effects (c) and (d) are considered in §§ 2.3.1 and 2.3.2, respectively.

Evans & Hochmuth (1976) applied a generalized Voigt–Kelvin stress–strain relation to simulate the viscoelastic response of the membrane. Puig-De-Morales-Marinkovic *et al.* (2007) found that the viscoelastic property of the RBC membrane followed a power law. Fractional order models were used by Craiem & Magin (2010) to study the viscoelasticity of RBCs. Lubarda (2011) presented a rate-type constitutive theory of elastic and viscoelastic response of an erythrocyte membrane for arbitrary isotropic strain energy functions. For simplicity, we use the generalized Voigt–Kelvin stress–strain relation by Evans & Hochmuth (1976) to model both the lipid bilayer and the cytoskeleton, which is written as

$$\Theta_1 h = \bar{T} + \frac{\mu_i}{2\lambda_1^2 \lambda_2^2} (\lambda_1^2 - \lambda_2^2) + 2\nu_i \frac{1}{\lambda_1} \frac{\mathrm{D}\lambda_1}{\mathrm{D}t}, \qquad (2.15)$$

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$$\Theta_2 h = \bar{T} + \frac{\mu_i}{2\lambda_1^2 \lambda_2^2} (\lambda_2^2 - \lambda_1^2) + 2\nu_i \frac{1}{\lambda_2} \frac{D\lambda_2}{Dt}, \qquad (2.16)$$

where D/Dt is the material derivative with respect to time t, and Θ_1 and Θ_2 are principal stresses. The constant v_i is the surface viscosity, and μ_i is the surface shear stiffness (i = b, s stands for the lipid bilayer or the cytoskeleton, respectively). Variable \overline{T} is the isotropic tension. For the cytoskeleton, μ_s and \overline{T} are calculated based on the molecular-detailed model of the JC, and the exact formulations are presented in Peng et al. (2010). For the lipid bilayer, $\overline{T} = K_b(\lambda_1\lambda_2 - 1)$, where $K_b = 5 \times 10^5$ pN μ m⁻¹ is the bilayer area stiffness, and λ_1 and λ_2 are principal stretches. Since the lipid bilayer is a fluid and its shear modulus is nearly zero, for numerical stability we choose a small but non-zero value as $\mu_b = 10^{-3}\mu_s$. The constant h is the thickness of the bilayer (2.2 nm) or the cytoskeleton (2 nm). The discrepancy between the bilayer thickness used herein and its actual value (4–5 nm) is attributed to the fact that in our study the bilayer is simplified as a continuous (but anisotropic) shell without considering its detailed molecular architecture. Detailed explanations of the bilayer and skeleton thicknesses used here can be found in Peng et al. (2010).

The viscosity for the lipid bilayer is given as $v_b = 10^{-9}$ Pa m s (Otter & Shkulipa 2007). Since the total membrane viscosity is measured as 5×10^{-8} Pa m s (Tran-Son-Tay, Sutera & Rao 1984), which is 50 times higher than the lipid bilayer viscosity, it is reasonable to assume that the membrane viscosity is mainly attributed to the viscosity of the cytoskeleton. Thus, we assign the viscosity of the cytoskeleton as $v_s = 5 \times 10^{-8}$ Pa m s.

Through numerical tests it was shown that for our modelling approach described in the previous sections, a direct numerical implementation of the Voigt–Kelvin stress–strain relation described in (2.15) and (2.16) may lead to numerical instability. To avoid this problem, we follow the numerical implementation of the viscous foam (material type 62) in the commercial finite element package LS-DYNA (Hallquist 1998) and incorporate an elastic term with a shear stiffness $\mu'_i = 100\mu_i$ in series with the viscous term (ν_i). Incidently, if $\mu'_i = \infty$ this formulation is exactly the same as the generalized Voigt–Kelvin model proposed by Evans & Hochmuth (1976). The numerical implementation of this viscoelastic model with finite strains can be found in Hallquist (1998) and Holzapfel (2000).

2.2.4. Bending stiffness of the lipid bilayer

The bending resistance of the lipid bilayer is included in the aforementioned continuum-based shell element by integrating the stress resultant in the thickness direction (Belytschko *et al.* 2000). Numerically, the bending moment is caused by the stress difference at the three Gaussian integration points in the thickness direction. It is different from traditional bending implementation by assigning a direct relationship between the bending moment and the curvature.

Considering a homogeneous shell with thickness h described by Evans & Skalak (1980), its bending stiffness k_c and area modulus K are related by

$$k_c = \int_{-(h/2)}^{+(h/2)} y^2 \frac{K}{h} \, \mathrm{d}y = \frac{Kh^2}{12}.$$
 (2.17)

For the lipid bilayer, we use h = 2.2 nm and $K = K_b = 5 \times 10^5$ pN μ m⁻¹ so that $k_c = 2 \times 10^{-19}$ J.

2.2.5. Boundary integral representation for fluid equations and boundary element discretization

By using the Lorentz reciprocal theorem (Pozrikidis 1992), (2.2) and (2.3) can be described by a boundary integral representation. In this study, we focus on the three-dimensional problem of an RBC immersed in shear flow in an open space, and the axisymmetric problem of a file of RBCs in a cylindrical tube.

For an RBC immersed in a shear flow, the boundary integral representation for the velocity at the point x_0 located in the exterior fluid (fluid 1) is given as (Pozrikidis 1992, 2003*b*)

$$\boldsymbol{v}^{f}(\boldsymbol{x}_{0}) = \bar{\boldsymbol{v}}^{f}(\boldsymbol{x}_{0}) - \frac{1}{8\pi\eta_{1}} \iint_{\Gamma^{fb}} \boldsymbol{G}(\boldsymbol{x}, \boldsymbol{x}_{0}) \cdot \Delta \boldsymbol{t}^{f}(\boldsymbol{x}) \, \mathrm{d}\Gamma(\boldsymbol{x}) + \frac{1 - \Lambda}{8\pi} \iint_{\Gamma^{fb}} \boldsymbol{v}^{f}(\boldsymbol{x}) \cdot \boldsymbol{T}(\boldsymbol{x}, \boldsymbol{x}_{0}) \cdot \boldsymbol{n}(\boldsymbol{x}) \, \mathrm{d}\Gamma(\boldsymbol{x}),$$
(2.18)

where $\bar{\boldsymbol{v}}^{f}(\boldsymbol{x}_{0})$ is the prescribed undisturbed velocity field of the shear flow in the absence of the cell, η_{1} is the viscosity of the exterior fluid and $\Lambda = \eta_{2}/\eta_{1}$. Vector $\Delta \boldsymbol{t}^{f} = \boldsymbol{t}^{f,1} - \boldsymbol{t}^{f,2}$ is the discontinuity in the interfacial surface traction, where $\boldsymbol{t}^{f,1}$ is the traction in the outside surface $\Gamma^{fb,1}$ of the interface and $\boldsymbol{t}^{f,2}$ is the traction in the inside surface.

The second term in the right-hand side of (2.18) is the single-layer potential, which represents contribution from the distribution of point forces associated with the Green's function for velocity. The third term is the double-layer potential, which represents contributions from point sources and point force dipoles. Matrix **G** contains the free-space Green's function for velocity G_{ii} expressed as

$$G_{ij}(\mathbf{x}, \mathbf{x}_0) = \frac{\delta_{ij}}{|\mathbf{x} - \mathbf{x}_0|} + \frac{(x_i - x_{0_i})(x_j - x_{0_j})}{|\mathbf{x} - \mathbf{x}_0|^3},$$
(2.19)

where δ_{ij} is Kronecker's delta. Matrix **7** is the Green's function for stress. Its components are

$$T_{ijk}(\boldsymbol{x}, \boldsymbol{x}_0) = -6 \frac{(x_i - x_{0_i})(x_j - x_{0_j})(x_k - x_{0_k})}{|\boldsymbol{x} - \boldsymbol{x}_0|^5}.$$
 (2.20)

As the point x_0 approaches the interface Γ^{fb} from the external side, we obtain a boundary integral equation as (Pozrikidis 1992, 2003*b*)

$$\boldsymbol{v}^{f}(\boldsymbol{x}_{0}) = \frac{2}{1+\Lambda} \bar{\boldsymbol{v}}^{f}(\boldsymbol{x}_{0}) - \frac{1}{4\pi\eta_{1}(\Lambda+1)} \iint_{\Gamma^{fb}} \boldsymbol{G}(\boldsymbol{x},\boldsymbol{x}_{0}) \cdot \Delta \boldsymbol{t}^{f}(\boldsymbol{x}) \,\mathrm{d}\Gamma(\boldsymbol{x}) + \frac{1-\Lambda}{4\pi(1+\Lambda)} \oint_{\Gamma^{fb}} \boldsymbol{v}^{f}(\boldsymbol{x}) \cdot \boldsymbol{T}(\boldsymbol{x},\boldsymbol{x}_{0}) \cdot \boldsymbol{n}(\boldsymbol{x}) \,\Gamma(\boldsymbol{x}), \qquad (2.21)$$

where $\int \int$ denotes the principal value integration.

Numerically, we apply the BEM to discretize the boundary integral equation (2.21). The collocation method is employed. A constant four-node quadrilateral element is developed. We discretize the boundary Γ^{fb} into N_{BE}^{fb} elements. The single-layer potential kernel with a weak 1/r ($r = |\mathbf{x} - \mathbf{x}_0|$) singularity is integrated by segmenting the quadrilateral into flat triangles and the integration is performed in a polar coordinate system. Four-by-four Gaussian integration points are employed to integrate (2.21) for both the quadrilateral element and the flat triangles. The weak $1/r^2$

singularity in the double-layer potential kernel is removed by using the relation

$$\int \int_{\Gamma^{fb}} \boldsymbol{v}^{f}(\boldsymbol{x}) \cdot \boldsymbol{T}(\boldsymbol{x}, \boldsymbol{x}_{0}) \cdot \boldsymbol{n}(\boldsymbol{x}) \, \mathrm{d}\Gamma(\boldsymbol{x}) \\
= \iint_{\Gamma^{fb}} [\boldsymbol{v}^{f}(\boldsymbol{x}) - \boldsymbol{v}^{f}(\boldsymbol{x}_{0})] \cdot \boldsymbol{T}(\boldsymbol{x}, \boldsymbol{x}_{0}) \cdot \boldsymbol{n}(\boldsymbol{x}) \, \mathrm{d}\Gamma(\boldsymbol{x}) + 4\pi \boldsymbol{v}^{f}(\boldsymbol{x}_{0}). \quad (2.22)$$

Applying the boundary integral equation at the collocation points of boundary Γ^{fb} , and after discretization of (2.21), we obtain $3N_{BE}^{fb}$ algebra equations, which is written symbolically in a matrix form as

$$\boldsymbol{v}_{BE}^{fb} = \bar{\boldsymbol{v}}_{BE}^{fb} - \boldsymbol{S}\boldsymbol{q}_{BE}^{fb} + \boldsymbol{D}\boldsymbol{v}_{BE}^{fb}, \qquad (2.23)$$

where v_{BE}^{fb} is the global vector including velocities at all collocation points on Γ^{fb} (i.e. its dimension is $3N_{BE}^{fb}$). Vector \bar{v}_{BE}^{fb} is the global vector of undisturbed velocities. Vector q_{BE}^{fb} is the global surface traction vectors on Γ^{fb} . Here **S** is the single-layer potential coefficient matrices on the interface Γ^{fb} . Matrix **D** is the double-layer potential coefficient matrix on the interface Γ^{fb} . The matrix vector multiplication Dv_{BE}^{fb} is achieved by using (2.22). Solving (2.23) for v_{BE}^{fb} , we obtain

$$\boldsymbol{v}_{BE}^{fb} = (\boldsymbol{I} - \boldsymbol{D})^{-1} [\bar{\boldsymbol{v}}_{BE}^{fb} - \boldsymbol{S} \boldsymbol{q}_{BE}^{fb}], \qquad (2.24)$$

where I is an identity matrix. For numerical efficiency, the solution of (2.24) is obtained by using successive substitutions instead of direct matrix inversion $(I - D)^{-1}$ (Pozrikidis 1992).

Now the interfacial velocity v_{BE}^{fb} is expressed in terms of q_{BE}^{fb} , which is transferred from finite elements of the lipid bilayer and is presented in § 2.2.6.

In a special case when $\Lambda = 1$ (i.e. the viscosities of the interior and the exterior fluids are identical), the computation is much simplified since (2.24) is reduced to

$$\boldsymbol{v}_{BE}^{fb} = \bar{\boldsymbol{v}}_{BE}^{fb} - \boldsymbol{S}\boldsymbol{q}_{BE}^{fb}.$$
 (2.25)

The problem of a file of periodic RBCs in a cylindrical tube with axisymmetric configuration can be formulated similarly by using a Green's function representing a periodic array of point force rings inside a circular cylinder, which is used in our simulations. The detailed form of this Green's function and related formulations can be found in Pozrikidis (1992, 2005). Six Gaussian integration points are used for the axisymmetric boundary elements, and uniformly reduced integration (Belytschko et al. 2000) is employed for the axisymmetric shell elements (i.e. one in-plane Gaussian integration point and three Gaussian integration points in the thickness direction).

2.2.6. Coupling FEM and BEM

According to the boundary conditions (2.6)-(2.8), the lipid bilayer and the fluid share the same velocity and balance the tractions on the interface. To achieve this, numerically we employ a staggered algorithm to couple FEM and BEM (Walter et al. 2010).

For the constant quadrilateral elements in BEM, four nodes are shared with the quadrilateral shell element derived in § 2.2.2. In axisymmetric cases, the axisymmetric Hughes–Liu shell elements (Hughes & Liu 1981a) are coupled with the axisymmetric boundary elements with the special Green's function mentioned in § 2.2.5 by sharing two nodes.

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Furthermore, it is necessary to relate the global nodal force and moment vectors f_{FE}^{fb} and m_{FE}^{fb} of finite elements to the surface traction vector q_{BE}^{fb} in boundary elements. Walter *et al.* (2010) employed iso-parametric elements for both FEM and BEM, and used membrane elements without bending stiffness to model the capsule. Based on the principle of virtual work, they related the nodal force f_{FE}^{fb} of membrane elements in FEM with the nodal traction q_{BE}^{fb} of BEM by solving a linear equation expressed as

$$\boldsymbol{M}\boldsymbol{q}_{\boldsymbol{B}\boldsymbol{E}}^{fb} = \boldsymbol{f}_{\boldsymbol{F}\boldsymbol{E}}^{fb}, \qquad (2.26)$$

where the square matrix M has a similar structure as the consistent mass matrix in FEM. However, for the continuum-based shell element with bending stiffness described in \S 2.2.2, there are also rotational degrees of freedom at the nodes, since it is based on the Reissner-Mindlin shell theory rather than the Kirchhoff-Love shell theory and the transverse shear deformation is allowed. The transverse shear stiffness acts as a penalty parameter as shown in Peng et al. (2010). The fibre can rotate locally due to the transverse shear deformation. Although the external nodal moments due to the traction may be small, they are required to calculate the local rotation of the fibre and cannot be neglected. If iso-parametric elements are used for both FEM and BEM, due to the external nodal moment, the system will be over-determined, i.e. there will be $3N_{FE}$ unknowns (q_{BE}^{fb}) but $6N_{FE}$ equations (f_{FE}^{fb}) and m_{FE}^{fb} . To deal with this problem, in our coupling algorithm we use constant value elements in BEM. Instead of solving (2.26), we employ a lumping technique for both the translational and rotational degrees of freedom. This method is similar to the mass lumping technique in the FEM with explicit time integration (Belytschko et al. 2000). It takes the external nodal moment into account when calculating the traction, and it also simplifies the numerical implementation by avoiding solving linear equations. The validation in 3.1 will show that this coupling algorithm is accurate in capturing both the in-plane membrane behaviour and the bending resistance, despite the fact that it does not strictly follow the principle of virtual work. Detailed description of this lumping technique is provided in Appendix.

Finally, the procedure of the staggered coupling algorithm is summarized as follows:

- (a) generate the FE and BE meshes based on the geometry;
- (b) get the internal forces and moments of the shell elements based on the deformation at time step n (described in §2.2.2) and the constitutive laws (described in §§2.2.3 and 2.2.4), and subtract external forces and moments from the skeleton-bilayer interaction (described in §2.3) to obtain the fluid-structure interaction forces and moments on the interface;
- (c) project the interaction forces and moments to surface tractions on the boundary elements using the lumping technique (described in Appendix);
- (d) apply (2.24) to obtain the velocities of the collocation points of the boundary elements (described in § 2.2.5); if $\Lambda \neq 1$, the method of successive substitutions is needed to solve the equations;
- (e) project the velocities of boundary element collocation points to finite element nodal velocities and calculate the local nodal angular velocities based on the external nodal moments (described in Appendix);
- (f) update coordinates at time step n + 1 using explicit time integration with finite element nodal velocities and update the fibre directions (described in Appendix);

(g) return to step (b) and repeat the calculation for the new configuration at time step n + 1.

It is noteworthy that although we obtain the nodal external forces and moments from the imposed displacements and rotations based on the configuration at time step n using the FEM, the displacement is solved at the new configuration at time step n + 1, which is consistent with the statement of the variational form (2.9).

2.3. 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 hydrodynamic interaction with the lipid bilayer *via* the cytoplasm. Hereby we only consider the hydrodynamic loads upon the cytoskeleton, whereas its influence on the surrounding flow field is not considered. Owing to the closeness of the cytoskeleton to the lipid bilayer, it is reasonable to assume that the cytoplasm near the lipid bilayer. In that sense, the interaction between the cytoskeleton and the cytoplasm can be merged with the cytoskeleton's interaction with the lipid bilayer. The balance of internal forces and external forces of the cytoskeleton leads to $\tau^{bs} + t_s = 0$, where τ^{bs} is the total interaction force per unit area between the lipid bilayer and the cytoskeleton (applied on the skeleton), and t^s is the internal force per unit area of the cytoskeleton due to its internal stress Θ^s . Vector t^s is calculated using the FEM, whereas Θ^s is presented in § 2.2.3.

For convenience we also use the shell element formulation presented in § 2.2.2 to model the cytoskeleton. One Gaussian integration point is used in the thickness direction so that the shell elements are actually reduced into membrane elements without bending stiffness. Thus, the rotational degrees of freedom of the shell elements do not need to be considered for the cytoskeleton. Numerically, t^s is calculated at the nodes *via* dividing the nodal forces by the nodal areas.

After t^s and τ^{bs} are obtained, the velocity of the cytoskeleton is calculated as follows. A local Cartesian system is defined so that z is in the normal direction of the cytoskeleton surface, x and y are tangential to the surface. The skeleton velocity, $v^s = [v^s_x v^s_y v^s_z]^T$, is obtained as

$$v_x^s = \frac{\tau_x^{bs}}{c_f + c_{xy}} + v_x^b, \tag{2.27}$$

$$v_{y}^{s} = \frac{\tau_{y}^{bs}}{c_{f} + c_{xy}} + v_{y}^{b},$$
(2.28)

$$v_z^s = v_z^b, \tag{2.29}$$

where $v^b = [v_x^b \ v_y^b \ v_z^b]^T$ is the velocity vector of the lipid bilayer, c_f is the viscous friction coefficient between the lipid bilayer and the cytoskeleton, which will be formulated in § 2.3.1, and c_{xy} and c_z are tangential and vertical hydrodynamic drag coefficients, which will be formulated in § 2.3.2. Strictly speaking, c_f is applied in front of the relative velocity between the skeleton and the lipid bilayer, whereas c_{xy} should be applied in front of the relative velocity between the skeleton and the skeleton and the cytoplasm. However, in the vicinity of the lipid bilayer these two are close to each other owing to the no-slip condition. For this reason in the current study we do not distinguish them. Equation (2.29) enforces the normal no-penetration condition between the lipid bilayer and the cytoskeleton. Numerically, v^s is calculated at the

node 'a' of the cytoskeleton mesh, while v^b is calculated at the projection point of node 'a' on the lipid bilayer mesh in the master-slave contact algorithm (Malone & Johnson 1994; Peng *et al.* 2010). Note that although τ_z^{bs} is not shown in (2.27)–(2.29), it can be obtained using $\tau^{bs} = -t_s$. Here τ_z^{bs} includes both the elastic interaction and the normal hydrodynamic interaction forces between the lipid bilayer and the cytoskeleton.

After the velocity of the cytoskeleton is obtained, the coordinates of the cytoskeleton are updated using explicit time integration (e.g. the explicit Euler method).

2.3.1. Viscous friction between the lipid bilayer and the cytoskeleton due to the mobilities of the anchored proteins

By applying the Stokes–Einstein relation, the drag force on a protein anchored in the lipid bilayer is given as

$$f = -\frac{v}{b_T} = -\frac{k_B T v}{D_T},\tag{2.30}$$

where v is the translational velocity of the protein. The minus sign refers to the fact that the drag force is in the opposite direction of the velocity. Constant D_T is the translational diffusivity of the protein. Constant b_T is the translational mobility of the protein. Constant $k_B = 1.38 \times 10^{-23}$ J K⁻¹ is the Boltzmann constant and T is the absolute temperature. The translational microscopic diffusivities of band 3 and glycophorin C in the lipid bilayer are measured experimentally as 0.0014–0.022 μ m² s⁻¹ (normal intact human RBCs) (Kodippili *et al.* 2009) and 4.0 μ m² s⁻¹ (Kapitza *et al.* 1984), respectively. Note that for band 3 two different diffusivities have been reported. One of them, the microscopic diffusivity, corresponds to a short time diffusion coefficient when band 3 only diffuses within one Sp compartment. The other, the macroscopic diffusivity, is a long time diffusion coefficient when band 3 can diffuse from one Sp compartment to another by crossing Sp barriers (Kodippili *et al.* 2009). In our study we use the microscopic diffusivity of normal intact RBCs, because when we model the bilayer-skeleton slip, we consider the motion of the whole JC including the band 3 so that the band 3 does not cross Sp barriers. In the following simulations we use the upper bound of the measured value of this property (i.e. 0.022 μ m² s⁻¹).

Thus, the drag forces on a band 3 and a glycophorin C are $f_B = -0.194 \text{ pN s } \mu\text{m}^{-1} \text{ V}$ and $f_G = -0.001 \text{ pN s } \mu\text{m}^{-1} \text{ V}$, respectively. The drag force on a glycophorin C is much smaller than that on a band 3.

In our problem, the translational velocity v of the proteins is equal to the relative tangential velocity between the lipid bilayer and the cytoskeleton. Considering 33 000 JCs with three band 3 (since the band 3's are shared by neighbouring JCs) and six glycophorin C per JC in a total area of 135 μ m² (the surface area of a normal human RBC) with a normal human temperature T = 310 K. The friction coefficient between the lipid bilayer and the cytoskeleton per unit area is then calculated as

$$c_f = -\frac{\rho}{\rho_0} \frac{(f_B/\nu + 2f_G/\nu) \times 3 \times 33\,000}{135\,\mu\text{m}^2} = \frac{\rho}{\rho_0} \cdot 144 \text{ pN s } \mu\text{m}^{-3}, \quad (2.31)$$

where ρ and ρ_0 are the current and initial protein densities of the cytoskeleton. The protein density is defined as the number of proteins per unit area. The factor ρ/ρ_0 is associated with cytoskeleton deformation and its effect upon the density of skeleton-bilayer pinning points (i.e. transmembrane proteins). This ratio also represents the density of skeleton-attached proteins, whose variations are experimentally measurable (see for example Discher *et al.* 1994). In practice, this density ratio is determined as $\rho/\rho_0 = 1/(\lambda_1\lambda_2)$, where λ_1 and λ_2 are principal in-plane stretches of the cytoskeleton. In our model, λ_1 and λ_2 are readily determined through FEM simulations of the inner layer (Peng *et al.* 2010).

For erythrocytes affected with the hereditary spherocytosis (spherocytes), Perrotta *et al.* (2005) found that the density of band 3 was $\sim 12 \pm 4\%$ of that of healthy RBCs (Butler, Mohandas & Waugh 2008, see also). Kodippili *et al.* (2009) found that the average microscopic diffusivity of the band 3 in human spherocytes is a little bit larger than those in normal human RBCs, but they are in the same order. According to (2.31) and (2.30), both decreased density and increased diffusivity of the band 3 will decrease the bilayer–skeleton friction coefficient. Based on the decreased band 3 density, we estimate the bilayer–skeleton friction coefficient in spherocytes as

$$c'_f = 10 \% c_f = \frac{\rho}{\rho_0} \cdot 14.4 \text{ pN s } \mu \text{m}^{-3}.$$
 (2.32)

Incidently, Sheetz, Schindler & Koppel (1980) showed that the translational macroscopic diffusivities of integral membrane proteins were increased by 50 times in mouse spherocytic RBCs. However, the macroscopic diffusivity depends significantly on protein–protein interactions. In addition, mouse RBCs may be different from human RBCs. Therefore, we do not use this value in our simulations.

Note that in these estimations we do not consider the fluid dynamics interactions among the transmembrane proteins as well as their interactions with the skeleton through the cytosol. This approximation is accurate when the protein density is low. To accurately account for these effects, in future work a three-dimensional fluid-structure interaction model for the interaction among the skeleton, the transmembrane proteins, the lipid bilayer and the cytosol is required.

2.3.2. Hydrodynamic drag on the cytoskeleton

Although the cytoskeleton is a porous network with three-dimensional structure, for computational efficiency when considering its interaction with the surrounding fluid we simplify it as a planar triangular network (i.e. only the Sps are considered). The overall drag is calculated as the summation of drag forces on each individual Sp, and the hydrodynamic interactions between Sps are not considered. Specifically, we consider each Sp as a cylindrical bar with length l and radius r immersed in infinite Stokes flow.

Hereby the drag coefficients are defined as $f_{\perp} = -c_{\perp}v_{\perp}$ and $f_{\parallel} = -c_{\parallel}v_{\parallel}$, where f_{\perp} is the transverse drag (i.e. the drag perpendicular to the cylinder axis), f_{\parallel} is the longitudinal drag (the drag parallel to the cylinder axis), v_{\perp} is the transverse velocity component, and v_{\parallel} is the longitudinal velocity component. If $\epsilon = r/l \ll 1$, then

$$c_{\perp} = \frac{4\pi\eta_2 l}{\ln\left(\frac{l}{r}\right) + \frac{1}{2}} [1 + O(\epsilon^2)], \qquad (2.33)$$

$$c_{\parallel} = \frac{2\pi\eta_2 l}{\ln\left(\frac{l}{r}\right)} [1 + O(\epsilon^2)], \qquad (2.34)$$

where $\eta_2 = 6 \text{ cP} = 0.006 \text{ pN} \text{ }\mu\text{m}^{-2} \text{ s}$ is the viscosity of the interior cytoplasm solution. Note that for $\epsilon \ll 1$, $c_{\perp} \sim 2c_{\parallel}$. For a Sp tetramer (i.e. two Sp dimers linked by head-to-head connection), the length is l = 75 nm and the radius is around r = 1 nm.

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Thus the drag coefficients are estimated as $c_{\perp} = 1.174 \times 10^{-3} \text{ pN s } \mu\text{m}^{-1}$ and $c_{\parallel} = 0.655 \times 10^{-3} \text{ pN s } \mu\text{m}^{-1}$.

We now consider a perfect triangle with length l on each side located inside the xy plane moving in a Stokes flow with a velocity $\boldsymbol{v} = [v_x, 0, v_z]$. The y direction is chosen to be in the triangle plane and perpendicular to the moving direction and z direction is chosen to be the normal direction to the triangle. We assume that the total drag on this triangle equals the sum of the drags on the three edges.

A simple calculation shows that the in-plane drag $f_{\Delta,x}$ of the triangle is isotropic (independent of the orientation of the triangle) and no lateral force (force in the y direction) is generated. The drags are calculated as $f_{\Delta,x} = -3/2(c_{\perp} + c_{\parallel})v_x = -(2.74 \times 10^{-3} \text{ pN } \mu\text{m}^{-1} \text{ s}) v_x$, $f_{\Delta,y} = 0$ and $f_{\Delta,z} = -3c_{\perp}v_z = -(3.52 \times 10^{-3} \text{ pN } \mu\text{m}^{-1} \text{ s})v_z$.

For a cytoskeleton network with a total area of $135 \,\mu\text{m}^2$, there are 33000 actin protofilaments. Each protofilament is in connection with six Sp tetramers, whereas each tetramer is connected with two protofilaments. Based on this, there should be 33000 independent triangles (such as that described above) in the network. Therefore, the tangential drag force of the network per unit area is

$$c_{xy} = -\frac{\rho}{\rho_0} \cdot 33\,000/135\,\,\mu\text{m}^2, \quad f_{\Delta,x}/v_x = \frac{\rho}{\rho_0} \cdot 0.67\,\,\text{pN s}\,\,\mu\text{m}^{-3},$$
 (2.35)

and the normal drag force of the network per unit area is

$$c_z = -\frac{\rho}{\rho_0} \cdot 33\,000/135\,\,\mu\text{m}^2, \quad f_{\Delta,z}/v_z = \frac{\rho}{\rho_0} \cdot 0.86\,\,\text{pN s}\,\,\mu\text{m}^{-3}.$$
 (2.36)

These hydrodynamic drags are included in τ^{bs} and transferred to the lipid bilayer through the boundary condition (2.8). Note that $c_f \gg c_{xy}$, i.e. the bilayer–skeleton viscous friction is much larger than the hydrodynamic drag in the tangential direction. Strictly, the wall effect of the lipid bilayer on the motion of Sps should be considered. In practice, however, since the hydrodynamic drag is much smaller than the bilayer–skeleton viscous friction, the current approach is sufficiently accurate.

3. Results

3.1. Model validations

The models at different levels in our multiscale approach have been tested extensively through comparisons with experiments as well as other theoretical and numerical studies. For example, the level I model has been validated by comparing the predicted tension-elongation curves of Sps with AFM measurements by Rief *et al.* (1999) (Zhu & Asaro 2008). The level II model of JC is tested by comparing the predicted orientations of the actin protofilaments and shear stiffness with various experiments (Zhu *et al.* 2007). In Peng *et al.* (2010), we tested the multiscale model by comparing with micropipette aspirations (see for example Waugh & Evans 1979) and optical tweezers experiments (see for example Dao *et al.* 2006). In those validations, we not only compared the overall cell deformation, but also the experimentally measured skeleton density variation (Discher *et al.* 1994). In addition, we have compared the resting cell shape with theoretical predictions by Seifert *et al.* (1991). In all of these cases our numerical predictions agree well with benchmark results.

The fluid-structure interaction model is validated by simulating two canonical cases: (a) RBCs passing through a cylindrical tube and (b) a spherical capsule and an RBC in shear flow with low shear rates. The predictions are then compared with benchmark results from previous studies. To be consistent with these existing



FIGURE 4. (a) The shape of RBCs passing through a cylindrical tube driven by flow. (b) Distributions of membrane tensions in meridional and azimuthal directions along the arc length (compared with Pozrikidis 2005). The tensions are normalized by ηU_m .

studies, in this validation work we consider a reduced version of our model: a singlelayer continuum model, in which the cell membrane is modelled as a single-layer structure (i.e. the detailed bilayer–skeleton architecture is not specified) with uniform mechanical properties. Furthermore, the mechanical parameters and the constitutive relations are kept the same as those in the corresponding previous studies to be compared with.

First, we simulate a file of RBCs passing through a cylindrical tube (which resembles cell motion inside capillaries) and compare our results with the predictions by Pozrikidis (2005). As shown in figure 4(a), the problem is considered to be axisymmetric with respect to the *x* axis (the centreline of the tube). Here *y* represents the distance measured from the centreline towards the boundary of the tube. As discussed in § 2.2.5, in axisymmetric cases the Green function is spatially periodic so that the method is capable of simulating an infinite sequence of cells without additional computational effort. The cell membrane is described by the Skalak law (Skalak *et al.* 1973) defined as

$$\Theta_1 h = \frac{G_{SK}}{\lambda_1 \lambda_2} \{ \lambda_1^2 (\lambda_1^2 - 1) + C(\lambda_1 \lambda_2)^2 [(\lambda_1 \lambda_2)^2 - 1] \},$$
(3.1)

$$\Theta_2 h = \frac{G_{SK}}{\lambda_1 \lambda_2} \{ \lambda_2^2 (\lambda_2^2 - 1) + C(\lambda_1 \lambda_2)^2 [(\lambda_1 \lambda_2)^2 - 1] \},$$
(3.2)

where G_{SK} is the shear modulus and C is a material coefficient. In the limit of small deformation, the area dilatation modulus K is given by (Barthès-Biesel, Diaz &



FIGURE 5. Schematic of an RBC (or capsule) in simple shear flow.

Dhenin 2002; Omori et al. 2011)

$$K = G_{SK}(1 + 2C). (3.3)$$

To validate our numerical algorithm by comparing results from Pozrikidis (2005), we use the same values of material properties as in Pozrikidis (2005), i.e. $G_{SK} =$ 2.1 pN µm⁻¹, C = 100.0 and bending stiffness $\kappa_c = 1.8 \times 10^{-19}$ J. We use L/a = 3.0, b/a = 1.0, G = 0.5, where L is the space between periodic cells (see figure 4a), $a = (3V/4\pi)^{1/3} \approx 2.82$ µm is the equivalent cell radius ($V \approx 98$ µm³ is the cell volume) and b is the tube radius. The undisturbed velocity profile \bar{v}^f is a parabolic function of y with the maximum speed is reached at y = 0. We define $G = \eta U_m/2G_{SK}$ as the reduced flow rate, where U_m is the maximum undisturbed flow velocity and $\eta = \eta_1 = \eta_2 = 1.2$ cP = 0.0012 pN µm⁻² s is the fluid viscosity.

The predicted shape of the RBCs is shown in figure 4(a), which is graphically indistinguishable from that obtained in Pozrikidis (2005) (see figure 8 in that paper). The distributions of membrane tensions in both the meridional and azimuthal directions along the ar clength *s* are shown in figure 4(b), which again demonstrate consistency with the study of Pozrikidis (2005). The arc length *s* is measured from the front point as shown in figure 4(a). The total arc length s_t is measured from the front point to the trailing point. The tensions are normalized by ηU_m .

Second, we simulate the motion of a spherical capsule in a simple shear flow with undisturbed velocity ky in the horizontal direction, where k is the shear rate and the centre of the cell lies at y = 0 (see figure 5). In this simulation, both the mechanical parameters ($G = \eta ka/\mu = 0.20$, where a is the equivalent radius and μ is the shear modulus of the membrane, and $\Lambda = 1$) and the constitutive law of the capsule with an energy function of a neo-Hookean form are taken from Ramanujan & Pozrikidis (1998). This constitutive law, as well as its related surface strain invariants, was originally derived by Barthès-Biesel & Rallison (1981). As shown in figure 6, our results, in this case for the inclination angle θ_{xy} of the maximum dimension with respect to the x axis in the xy plane (the mid-plane) and the Taylor deformation parameter $D_{xy} = (A - B)/(A + B)$ (A, B are the maximum and minimum dimensions in xy plane; see figure 5 for definitions of θ_{xy} , A, and B), match well with the reported data in Ramanujan & Pozrikidis (1998). In practice, to calculate A and B we adopt the approach suggested by Ramanujan & Pozrikidis (1998). The basic procedure is: (a) calculate the inertia tensor of the cell or the capsule; (b) calculate the dimensions



FIGURE 6. (a) The inclination angle and (b) the Taylor deformation parameter as functions of time.



FIGURE 7. Cell shapes (top) and profiles in the *xy* plane (bottom) at (*a*) kt = 0, (*b*) kt = 4, (*c*) kt = 8.15 and (*d*) kt = 11.80.

of an equivalent triaxial ellipsoid with the same inertia tensor; (c) calculate A and B of this ellipsoid, and use these to find D_{xy} of the cell.

Finally, we simulate the tumbling motion of an RBC in a shear flow by incorporating both bending stiffness and area stiffness of its membrane, and compare our results with those in Pozrikidis (2003*b*). The constitutive law used here is the same as in Pozrikidis (2003*b*), which is similar to that for capsules in Ramanujan & Pozrikidis (1998) but with an extra area stiffness term. The reduced shear rate G = 0.1, the bending stiffness $k_c = 1.8 \times 10^{-19}$ J, the shear modulus $\mu = 3$ pN µm⁻¹, and the normalized area stiffness $K/\eta_1 ka = 200$, where K is the area stiffness. Following Pozrikidis (2003*b*), we use A = 5 ($\eta_2 = 0.006$ pN µm⁻² s). Figure 7 shows snapshots of the cell motion/deformation as well as the cell profiles in the *xy* plane. These results are very close to the predictions by Pozrikidis (2003*a*,*b*) (see figure 4*a* in that paper). The corresponding time evolution of the inclination angle is plotted in figure 8, which agrees well with Pozrikidis (2003*b*).

To further illustrate the accuracy of the bending resistance in our coupling algorithm, we consider another case with a smaller bending stiffness $k_c = 4.5 \times 10^{-20}$ J. Other parameters remaining unchanged. The time evolution of the inclination angle in this case is also plotted in figure 8. It is seen that with a smaller bending stiffness, the result becomes significantly different.

In addition, we also compare our double-layer multiscale model with the singlelayer continuum model for tumbling motion as shown in figure 8. To compare with the result by Pozrikidis (2003*b*), we scale the initial shear modulus of our multiscale model to $\mu = 3 \text{ pN } \mu\text{m}^{-1}$. We consider two cases in the multiscale model: one with membrane viscosity and the other without membrane viscosity. It is seen the tumbling



FIGURE 8. Evolution of the inclination angle θ_{xy} during the tumbling motion of an RBC. The result is compared with the prediction by Pozrikidis (2003*b*).

rate of our multiscale model is a little bit faster than the simple continuum model due to the strain stiffening effect. Furthermore, the effect of membrane viscosity on tumbling motion is small.

The multiscale structural model has been validated extensively in Peng et al. (2010), in which it was applied to simulate quasi-static responses of RBC induced by optical tweezers or micropipettes (micropipette aspirations). In optical tweezers experiments, a pair of beads are attached to the cell (one on each side). The motions of these beads are controlled by laser beams, and the stretching force applied by these beads can be experimentally calibrated. Through systematic measurements (Dao et al. 2006), the cell deformation has been correlated with the force imposed by the tweezers. In micropipette aspirations, an RBC is partially sucked into a micrometresize pipe (i.e. a micropipette) with negative pressure inside it. The length of the cell entering the pipette is linearly related with the imposed pressure (see for example Waugh & Evans 1979). With fluorescent protein marking, the density variation of the cytoskeleton (which represents area deformation) was also measured (Discher et al. 1994). A coarse-grained model has been developed to duplicate the observed skeleton deformation (Discher et al. 1998). With our multiscale model, we have studied overall cell deformation as well as area and shear deformation of the cytoskeleton in both cases. All of our predictions are quantitatively consistent with the aforementioned experimental measurements and numerical simulations.

In the following we apply the fluid-structure coupled multiscale model to examine the deformation of the cytoskeleton (which is related to the mechanical loads inside it), and the interaction forces between the lipid bilayer and the skeleton in both tangential and normal (i.e. vertical with respect to the lipid bilayer) directions in the aforementioned scenarios associated with Stokes flows (inside a tube and a shear flow). We also study the area deformation and the shear deformation of the skeleton.

3.2. Cytoskeleton deformation and internal force inside an RBC in a tube

Using our multiscale model, we herein predict the protein density of the skeleton and the interaction force between the lipid bilayer and the skeleton in RBC membranes in a tube flow (figure 9b). The cell profile, as well as the surrounding flow fields

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inside and outside of the cells, is shown in figure 9(*a*). For flow conditions, we use the same parameters as in § 3.1, e.g. L/a = 3.0, b/a = 1.0, G = 0.5 and $a = 2.82 \,\mu\text{m}$. The parameters of the cytoskeleton, including the persistence lengths of folded and unfolded domains in Sp, the contour lengths of the folded and unfolded domains, the difference between the activation length of the unfolding process and that of the refolding process, and the force corresponding to the state when half of the domains are unfolded, are obtained from Peng *et al.* (2010). Lac & Barthès-Biesel (2005) and Lefebvre & Barthès-Biesel (2007) showed that the membrane prestress plays an important role for RBC motions in both tube flow and shear flow. To match the skeleton density variation recorded in micropipette aspirations, the prestress of the cytoskeleton is set to be $\bar{T}_0 = \bar{T}|_{\lambda_1=1,\lambda_2=1} = -30 \text{ pN }\mu\text{m}^{-1}$ (Peng *et al.* 2010). The spontaneous curvature of the bilayer is assumed to be zero, i.e. in its unloaded state a piece of lipid bilayer remains flat. Since in this particular case we only focus on the final steady configuration, the membrane viscosity, the bilayer–skeleton friction and the plasma viscosity contrast are irrelevant.

Figure 9(b) shows the distribution of the density ratio along the arclength s. It is seen that the skeleton is expanded ($\rho/\rho_0 < 1$) at the head region (the head of the bullet shape) and the trailing region (the bottom), whereas it is compressed ($\rho/\rho_0 > 1$) at the side of the cell that is almost parallel to the tube wall (0.3 < s < 0.6). A slight variation of the density ratio occurs at the edge formed between the bottom and the side ($s \sim 0.7$). The increase of protein density near the wall (0.3 < s < 0.6, e.g. point B) as shown in figure 9(a) and (b) might help facilitate the biochemical interaction between the RBC cytoskeleton and the endothelial cells on the vessel wall. Indeed, it has been showed that RBCs can release nitric oxide (NO) (Kleinbongard *et al.* 2009) to dilate the blood vessel and improve blood perfusion, as well as adenosine triphosphate (ATP) to regulate blood pressure (Wan, Ristenpart & Stone 2008).

The normal interaction force between the lipid bilayer and the skeleton is also plotted in figure 9. No tangential force exists since the sliding between the lipid bilayer and the cytoskeleton has been already finished before this final steady configuration. The quantity we show is the interaction force applied on one JC, which is obtained as the product between the interaction force per unit area τ^{bs} and the area of one JC after the deformation of the skeleton in that particular location as

$$f_{jc} = \frac{135 \text{ cm}^2}{33\,000} \frac{\rho_0}{\rho} \boldsymbol{\tau}^{bs}.$$
(3.4)

This force is negative when 0 < s < 0.8 (a negative normal interaction force refers to the scenario when the skeleton and bilayer tend to separate from each other (dissociation tendency)). The maximum negative interaction force occurs at the trailing edge of the bullet shape as shown as point A in figures 9(a) and (b). For 0.8 < s < 1, this force is positive, i.e. the skeleton and bilayer are pushed towards each other (association tendency).

A noteworthy phenomenon is that in tube flow (capillary flow) both the protein density variation and the bilayer-skeleton interaction forces are much lower than those during the micropipette aspiration experiments. During micropipette aspirations, the maximum interaction force per JC (i.e. the force that induces bilayer-skeleton separation) is 5–20 pN as shown in Peng *et al.* (2010), whereas in capillary flow the value is less than 0.3 pN. This may help explain the structural stability and durability of RBCs when they pass through the capillaries.

In addition, we also carried out a comparison between our multiscale model and the aforementioned single-layer continuum model. In this case, for the single-layer



FIGURE 9. (a) The cell profile and the flow field, (b) the protein density ratio ρ/ρ_0 and the normal interaction force acting on each JC when RBCs passing through a cylindrical tube and (c) comparison of shear deformation between the multiscale model and the single-layer continuum model.

continuum model, we employ the constitutive law as shown in (2.15) without viscosity. The shear modulus $\mu = 6 \text{ pN } \mu \text{m}^{-1}$ and the area modulus $K = 5 \times 10^5 \text{ pN } \mu \text{m}^{-1}$ are obtained from the literature (Mohandas & Evans 1994).

In figure 9(c), we compare the shear deformations predicted by the multiscale model and this continuum model. The magnitudes of the shear ratio are in the same range, but the distribution of the shear ratio shifts. The bilayer-skeleton interaction force cannot be obtained by using this single-layer model. In addition, in this single-layer model the protein density is constant due to the areal incompressibility $(K \gg \mu)$.

In these axisymmetric simulations of capillary flow, 100 linear axisymmetric shell elements were used for the lipid bilayer, 100 linear axisymmetric shell elements were used for the cytoskeleton and 100 constant boundary elements were used for the lipid bilayer. Time step size is 2×10^{-7} s and it takes 0.015 s to reach the steady-state shape starting from a biconcave shape. Such a case takes several hours of computational time to simulate on a single Intel 2.5 GHz CPU.

3.3. Tank-treading motion in shear flow

In § 3.1, we discussed the tumbling motion of RBC in shear flow. When the shear rate is sufficiently large, however, these cells may demonstrate a new type of response called tank-treading motion, in which a RBC deforms to an ellipsoidal shape and the membrane circulates around while the inclination angle remains almost unchanged.

To simulate this tank-treading response, we use the same parameters as in § 3.2. The viscosity of the internal fluid $\eta_2 = 6 \text{ cP} = 0.006 \text{ pN} \text{ }\mu\text{m}^{-2} \text{ s.}$

In figure 10, we compare the predicted tank-treading frequency (defined as the inverse of the period for a point on the membrane to complete one circle around the cell) with the experimental measurements by Fischer, Stöhr-Liesen & Schmid-Schönbein (1978). We show the relations between shear rate and tank-treading frequency with external fluid viscosities 13, 31 and 59 cP. It is seen that the tank-treading frequency increases linearly with the shear rate. Furthermore, our results demonstrate an increase in the ratio of tank-treading frequency to shear rate when the external fluid viscosity is increased. These features are consistent with experiments by Tran-Son-Tay (1983) and Fischer (2007), and the numerical results by Dodson & Dimitrakopoulos (2010). If the membrane viscosities are neglected ($v_b = v_s = 0$), then the frequency is overestimated as shown in figure 10. This is consistent with the result by Fedosov *et al.* (2010). All of the data points from the simulations are shown in circles.

We also compare tank-treading motions predicted by our multiscale model with those predicted by the single-layer continuum model with constitutive law as shown in (2.15) ($\mu = 6 \text{ pN } \mu \text{m}^{-1}$ and $K = 5 \times 10^5 \text{ pN } \mu \text{m}^{-1}$). The difference in tank-treading frequency between predictions of the multiscale model and the single-layer continuum model is small.

In the following simulations we consider two typical *in vivo* shear rates, 270 and 1640 s^{-1} ; 270 s⁻¹ is considered as the average shear rate and 1640 s^{-1} is considered as the peak shear rate in human body (Stroeva, Hoskinsb & Eassona 2007) or in an artificial heart (Hochareon 2003; Deutsch *et al.* 2006). Our results show that for both shear rates, the areal dilatation and protein density variation of the cytoskeleton are less than 3% everywhere if the friction coefficients derived in § 2.3 are used. This is in contradiction with simulation results by Dodson & Dimitrakopoulos (2010), in which measurable cytoskeleton areal dilatation was predicted. The primary cause of this discrepancy is the inclusion of dissipation effects. Specifically, in the model by Dodson & Dimitrakopoulos (2010), neither the bilayer–skeleton friction nor the



FIGURE 10. The tank-treading frequency as a function of the shear rate for different blood plasma viscosities and membrane viscosities. The results are compared with experimental measurements by Fischer *et al.* (1978). The data points from the simulations to construct the lines are shown in circles.

membrane viscosity is considered. Our simulations confirm the prediction by Fischer (1992) that during normal physiological conditions the tank-treading motion is too fast to allow significant bilayer–skeleton slip.

In figure 12, we compare the area variation of our multiscale model with Dodson & Dimitrakopoulos (2010) at a low shear rate $k = 22.16 \text{ s}^{-1}$. Following Dodson & Dimitrakopoulos (2010), we simulate an RBC in shear flow with capillary number $Ca = \eta_1 ka/\mu_s = 1.5$, $\Lambda = 0.1$, $\mu_s = 2.5$ pN μ m⁻¹ and $\eta_1 = 0.006$ pN μ m⁻² s. It is seen that the areal variation of the middle point on the upper dimple (point M as shown in figure 5) is much smaller than that obtained by Dodson & Dimitrakopoulos (2010). Note that we herein used the upper bound value of the microscopic diffusivity measured by (Kodippili et al. 2009). If we use lower bound value, the areal variation is even smaller. On the other hand, if we reduce the bilayer-skeleton friction coefficient by 10 times, the agreement with Dodson & Dimitrakopoulos (2010) is greatly improved. This may be the case for the hereditary spherocytosis, since Perrotta et al. (2005) found that the density of band 3 was $\sim 12 \pm 4\%$ of that of healthy RBCs (Butler et al. 2008, see also). Kodippili et al. (2009) found that the average microscopic diffusivity of the band 3 in human spherocytes was slightly larger than that in normal human RBCs (the two are of the same order of magnitude). According to (2.31) and (2.30), both decreased density and increased diffusivity of the band 3 will decrease the bilayer-skeleton friction coefficient. The corresponding bilayer-skeleton friction coefficient c_f is estimated as 10% of the friction coefficient c_f of healthy RBCs as shown in §2.3.1. The significant area variation of the case for the hereditary spherocytosis in figure 12 may help explain the hypothesis of the lipid bilayer loss due to significantly decreased protein density in the hereditary spherocytosis (Walensky et al. 2003).

Our simulations also demonstrate swinging motions of the cell, referring to variations of the inclination angle θ_{xy} and the cell shape (characterized by the Taylor deformation parameter D_{xy}) over time. The time histories of θ_{xy} and D_{xy} at shear rates of 270 s⁻¹ and 1640 s⁻¹ are shown in figure 11. For the shear rate of 270 s⁻¹, the time period of swinging motion is found to be 0.08 s, which is half of the time period of



FIGURE 11. Time histories of θ_{xy} and D_{xy} during swinging motions: (a) $k = 270 \text{ s}^{-1}$, $\eta_1 = 13 \text{ cP}$; (b) $k = 1640 \text{ s}^{-1}$, $\eta_1 = 13 \text{ cP}$.



FIGURE 12. Time evolution of the area variation of the middle point on the upper dimple (point M as shown in figure 5). The results are compared with the prediction by Dodson & Dimitrakopoulos (2010) ($Ca = \eta_1 ka/\mu_s = 1.5$ and $\Lambda = 0.1$).

the tank-treading motion (0.16 s) (figure 10). This is consistent with the conclusions by Ramanujan & Pozrikidis (1998) and Fedosov *et al.* (2010).

Contours of the shear ratio $\sqrt{\lambda_1/\lambda_2}$ as well as the tangential and normal interaction forces between the lipid bilayer and the cytoskeleton are shown in figure 13. The shear rate is 270 s⁻¹, and the external fluid viscosity $\eta_1 = 13$ cP. The corresponding vector field of the tangential interaction forces (the interaction forces on the lipid bilayer) is shown in figure 14. The in-plane flow velocity field within the *x*-*y* cross section is shown in figure 15. Note that on the cell surface the streamlines are not exactly coincident with the cell profile because there is a swinging motion. The flow velocity field is continuous across the membrane due to the no-slip condition, whereas the stress field is discontinuous across the membrane (shown as Δt^f in (2.18)). The corresponding contours for shear rate 1640 s⁻¹ are shown in figure 16. Incidently, the



FIGURE 13. Contours (top view and side view) of (a) the shear ratio $\sqrt{\lambda_1/\lambda_2}$, (b) magnitude contour of the tangential interaction force per JC and (c) magnitude contour of the normal interaction force per JC. $k = 270 \text{ s}^{-1}$, $\eta_1 = 13 \text{ cP}$. Contours are shown at the instant when D_{xy} reaches the maximum value.

cell shapes shown in figures 13 and 16 match well with the experimental pictures as shown in figure 4.4.2 in Pozrikidis (2003a).

The results show that in both cases the minimum shear ratios happen at the tips of the cell and their values are close to 1. The maximum shear ratio is 1.589 for shear rate 270 s⁻¹ and 2.120 for shear rate 1640 s⁻¹, occurring near the middle region of the cell but off from the centre plane (the *xy* plane).

For the tangential interaction force, the maximum values occur at the tips of the cell (0.042 pN per JC for shear rate 270 s^{-1} and 0.313 pN per JC for shear rate 1640 s^{-1}). In the top view of the vector field (figure 14) it is seen that there are points (P and Q) where the tangential interaction force changes direction. In figure 14, the bilayer–skeleton interaction force on the lipid bilayer instead of on the skeleton is shown for clarity.



FIGURE 14. Vector field of the tangential interaction force applied on the lipid bilayer, $k = 270 \text{ s}^{-1}$, $\eta_1 = 13 \text{ cP}$. It is shown at the time when D_{xy} reaches the maximum value.

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FIGURE 15. The flow velocity field around the cell,  $k = 270 \text{ s}^{-1}$ ,  $\eta_1 = 13 \text{ cP}$ . It is shown at the time when  $D_{xy}$  reaches the maximum value.

For normal interaction forces, the maximum value again occurs at the tips of the cell (0.182 pN per JC for shear rate 270 s⁻¹ and 0.551 pN per JC for shear rate 1640 s⁻¹). Since we consider pre-compression of the cytoskeleton with prestress  $\bar{T}_0 = -30$  pN  $\mu$ m⁻¹ (Peng *et al.* 2010), the cytoskeleton pushes the lipid bilayer outwards. The normal interaction forces mainly depend on the prestress and the curvature of the shape.

The distributions of the shear ratio and interaction forces along the cell profile within the xy plane are shown in figures 17-19 for shear rates  $270 \text{ s}^{-1}$  and  $1640 \text{ s}^{-1}$ . In figure 18, positive value indicates that the force is towards the x direction and negative value indicates that the force is towards the -x direction.

We also compare our multiscale model with the aforementioned single-layer continuum model with constitutive law as shown in (2.15) ( $\mu = 6 \text{ pN} \text{ }\mu\text{m}^{-1}$  and  $K = 5 \times 10^5 \text{ pN} \text{ }\mu\text{m}^{-1}$ ) for shear deformation with shear rate 270 s⁻¹ as shown in figure 17. The distribution of the shear deformation is similar, whereas the magnitude of the shear ratio is slightly smaller in the multiscale model. This difference is likely to be attributed to the strain-stiffening effect of the cytoskeleton included in our multiscale model.

In the simulation of shear flow with shear rate  $270 \text{ s}^{-1}$ , 1250 quadrilateral shell elements were used for the lipid bilayer, 1250 quadrilateral shell elements were used for the cytoskeleton and 1250 quadrilateral constant boundary elements were used for the lipid bilayer. In the simulation of shear flow with shear rate 1640 s⁻¹, 3000 quadrilateral shell elements were used for the lipid bilayer, 3000 quadrilateral shell elements were used for the lipid bilayer.



FIGURE 16. Contours (top view) (a) the shear ratio  $\sqrt{\lambda_1/\lambda_2}$ , (b) magnitude contour of the tangential interaction force per JC and (c) magnitude contour of the normal interaction force per JC. Here  $k = 270 \text{ s}^{-1}$ ,  $\eta_1 = 13 \text{ cP}$ . Contours are shown at the time when  $D_{xy}$  reaches the maximum value.



FIGURE 17. Shear ratio in the cross section by the xy plane (shown at the time when  $D_{xy}$  reaches the maximum value).

elements were used for the lipid bilayer. A typical case takes around several days to simulate on a single 2.5 GHz Intel CPU. A typical time step size is  $2 \times 10^{-6}$  s.

To better understand the distributions of the tangential and normal interaction forces, we consider an infinitely small element of the cytoskeleton along the centre line (the xy cross section) as shown in figure 20. The equilibriums of the cytoskeleton (a membrane without bending stiffness) in the tangential direction and the normal



FIGURE 18. Tangential interaction force per JC in the cross section by the xy plane (shown at the time when  $D_{xy}$  reaches the maximum value). Positive value indicates that the force is towards the x direction and negative value indicates that the force is towards the -x direction.



FIGURE 19. Normal interaction force per JC in the cross section by xy plane (shown at the time when  $D_{xy}$  reaches the maximum value).

direction (the Laplace's law) lead to

$$\frac{\partial T_x}{\partial s} + f^{bs} = 0, \tag{3.5}$$

$$\frac{T_x}{r_x} + \frac{T_z}{r_z} + p^{bs} = 0, ag{3.6}$$

where  $T_x = \Theta_x h$  and  $T_z = \Theta_z h$ ,  $r_x$  and  $r_z$  are the radii of the curvatures in x and z directions, respectively,  $f^{bs}$  is the friction force per unit area (the component of  $\tau^{bs}$  in the tangential direction) and  $p^{bs}$  is the normal interaction force per unit area (the component of  $\tau^{bs}$  in the normal direction).



FIGURE 20. The equilibrium of an infinitely small element of the cytoskeleton at the centre line (cross section by the *xy* plane).

There is almost no area change  $(\lambda_1 \lambda_2 \approx 1)$  in the cytoskeleton so that the mean stress due to area change is extremely small  $(\bar{T} \approx \bar{T}_0)$  and the shear ratio  $\gamma = \sqrt{\lambda_1/\lambda_2} \approx \lambda_1$ . Here  $\bar{T}_0 = -30 \text{ pN } \mu\text{m}^{-1}$  is the prestress. If we ignore the cytoskeleton viscosity temporarily  $(\nu_s = 0)$ ,  $T_x$  and  $T_z$  can be written as

$$T_x = T_0 + T_{shear},\tag{3.7}$$

$$T_z = T_0 - T_{shear},\tag{3.8}$$

where  $T_{shear} = 0.5\mu_s(\gamma^2 - 1/\gamma^2)$  is the shear stress as shown in (2.15) and (2.16). Therefore (3.5) can be re-expressed as

$$f^{bs} = -\frac{\partial T_{shear}}{\partial s} = -\frac{\partial T_{shear}}{\partial \gamma} \frac{\partial \gamma}{\partial s} = -\frac{\mu_s}{2} \left( 2\gamma + \frac{2}{\gamma^3} \right) \frac{\partial \gamma}{\partial s}.$$
 (3.9)

Equation (3.9) relates the friction force  $f^{bs}$  to the derivative of  $\gamma$  with respect to the arc length *s*. This relation is demonstrated in figures 17 and 18. Indeed, it can be shown that there are two points where the derivative of  $\gamma$  with respect to the arc length *s* equals to zero in figure 17, corresponding to two points (P and Q) in figure 18 where the tangential force equals zero. The drifts of the two points in arclength positions from figure 17 to figure 18 are caused by the cytoskeleton viscosity ( $\nu_s \neq 0$ ).

For normal interaction forces, if we ignore the cytoskeleton viscosity temporarily  $(v_s = 0)$ , (3.6) can be rewritten as

$$\bar{T}_0\left(\frac{1}{r_x} + \frac{1}{r_z}\right) + T_s\left(\frac{1}{r_x} - \frac{1}{r_z}\right) + p^{bs} = 0.$$
(3.10)

This relation explains characteristics of figure 19. At the convex tips the curvatures  $(1/r_x \text{ and } 1/r_z)$  are significantly larger than those in other places on the surface and  $T_s$  is very small due to small shear ( $\gamma \approx 1$ ), therefore the maximum normal interaction force  $p^{bs}$  happens in the tips.

Although the cell shapes predicted in figures 13 and 16 match well with the experiments, our simulations show significant swinging motions, while there is no significant swinging motion based on the experimental pictures by Fischer *et al.* (1978) and figure 4.4.2(b) of Fischer in Pozrikidis (2003*a*). A possible cause of this discrepancy is the choice of the reference configuration of the cytoskeleton, i.e. the configuration with zero shear deformation. In our simulations, we chose the biconcave shape as the reference configuration, but existing studies (e.g. Lim, Wortis & Mukhopadhyay 2002) suggest that the actual reference configuration may be between the biconcave shape and the spherical shape. By deflating a spherical cell into a biconcave shape first as we did in our previous work (Peng *et al.* 2010), we simulate the tank-treading motion of this RBC with a spherical shape as its reference

configuration. It is found that in this scenario there is almost no swinging motion. This confirms the work by Tsubota & Wada (2010). On the other hand, the reference shape may not be a perfect sphere for *in vivo* RBCs. According to the experiment by Fischer (2004), RBCs have shape memory. This implies that the reference shape of the cell is not perfectly spherical (otherwise all points on the membrane will be indistinguishable so that there will be no shape memory). Thus, our results provide indirect evidence that the reference configuration is between the biconcave shape and the spherical shape.

# 4. Conclusions and discussion

The vital difference between our model and the existing models is the incorporation of a multiscale structural model to describe the mechanical response of the cell membrane, which enables predictions of not only highly accurate RBC responses to external loads, but also the physical mechanisms involved in the dynamic response of a cell at different temporal and spatial levels. The multiscale simulations illustrated novel (and potentially important) phenomena in membrane mechanics that had never been discovered using other models.

Compared with the continuum models, our multiscale approach does not require a significantly larger computational cost. This is due to the fact that it is a hierarchical information-passing multiscale algorithm, in which different models at different length scales are simulated independently. The results from the lower level models (levels I and II) are precalculated and used as a 'database' for the upper level model. It is different from the concurrent multiscale algorithm, in which models at different levels are simulated simultaneously at different regions so that computational cost is usually very high. In addition, the computational cost of a double-layer model is almost the same as a single-layer model, because the dominant cost is the boundary element part of the lipid bilayer and the computational cost on the cytoskeleton is almost negligible.

Among the important phenomena that have been analysed are the remodelling of protein density and the development of both positive (tensile) and negative (compressive) forces that act between the lipid bilayer and the attached skeleton. For example, in our previous investigations the development of negative contact forces between the skeleton and the lipid bilayer, coupled with protein density changes, was related to the phenomena of membrane necking and vesiculation during RBC aspiration into a micropipette (Peng et al. 2010). During aspiration, however, large variations in skeleton density are typically forecast as, in fact, found experimentally. Density variations are easily in the range  $0.2 < \rho/\rho_0 < 1.4$ . Accompanying this is the development of negative contact force (per JC) as large as  $\sim -20$  pN. In contrast, we show here that within tube flow and shear flow RBCs undergo quite modest protein density variations as illustrated in the example simulation results shown in figure 9 where  $0.85 < \rho/\rho_0 < 1.07$ . Likewise the local contact forces per JC are also modest. The implication is that micropipette aspirations may exaggerate the mechanical loading on RBC and the subsequent mechanical responses in most in vivo conditions (e.g. inside capillaries or blood vessels).

On the other hand, RBCs may sustain much larger loads and deformations inside spleen, where blood flows from the red-pulp cords (ends of the small arterioles) to the venous sinuses and merges back into the venous system (Mebius & Kraal 2004). The venous sinuses are made of parallel series of endothelial cells with slits between them. Normal RBCs can pass through these slits, while aging, defected, or infected RBCs may be stuck there, where they are phagocytosed by macrophages. Furthermore, the

contractility of the stress fibres in the endothelial cells can control the opening of these slits and assist the retention of RBCs in the spleen. Further studies are necessary to illustrate these processes.

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# Appendix. FEM-BEM coupling algorithm

We consider a node 'a' with nodal force  $f^{(a)}$  and moment  $m^{(a)}$ , which is connected with *n* elements with indexes i = 1, ..., n. To conserve the linear momentum, we distribute finite element nodal forces  $f^{(a)}$  to the connected neighbouring elements as surface tractions based on the weights of element areas, i.e.  $\bar{q}_i^{(a)} = f^{(a)}/A^{(a)}$ , where  $\bar{q}_i^{(a)}$ is the surface traction on element *i* from node 'a' due to its nodal force, and  $A^{(a)}$  is the area sum of connected elements on node 'a'.

To conserve the angular momentum, we distribute the nodal moments to the neighbouring elements as force couples. Let  $r_i$  denote the relative position vector from the node 'a' to the collocation point (the centroid) of the element *i*, the moment  $m_i^{(a)}$  assigned to element *i* as

$$\boldsymbol{m}_{i}^{(a)} = \boldsymbol{R}_{i}^{2} \cdot \boldsymbol{W}^{-1} \cdot \boldsymbol{m}^{(a)}, \qquad (A 1)$$

where

$$\boldsymbol{R}_{i} = \begin{bmatrix} 0 & -\hat{r}_{i,3} & \hat{r}_{i,2} \\ \hat{r}_{i,3} & 0 & -\hat{r}_{i,1} \\ -\hat{r}_{i,2} & \hat{r}_{i,1} & 0 \end{bmatrix},$$
(A2)

$$\hat{\boldsymbol{r}}_i = \boldsymbol{r}_i - \frac{1}{n-1} \sum_{j=1(j\neq i)}^n \boldsymbol{r}_j, \qquad (A3)$$

and

$$\boldsymbol{W} = \sum_{i=1}^{n} \boldsymbol{R}_{i}^{2}, \qquad (A4)$$

where  $\hat{r}_{i,1}$ ,  $\hat{r}_{i,2}$  and  $\hat{r}_{i,3}$  are the three components of the vector  $\hat{r}_i$ .

We note that  $\sum_{i=1}^{n} \boldsymbol{m}_{i}^{(a)} = \boldsymbol{m}^{(a)}$  and  $\boldsymbol{m}_{i}^{(a)} \cdot \hat{\boldsymbol{r}}_{i} = 0$ , i.e.  $\boldsymbol{m}_{i}^{(a)}$  is perpendicular to  $\hat{\boldsymbol{r}}_{i}$ , which guarantees the existence of an equivalent force couple for  $\boldsymbol{m}_{i}^{(a)}$  associated with position vector  $\hat{\boldsymbol{r}}_{i}$ . This equivalent force couple is obtained by applying a force  $f_{i}^{(a)'} = \boldsymbol{R}_{i} \cdot \boldsymbol{W}^{-1} \cdot \boldsymbol{m}^{(a)}$  at the centre of this element while applying opposite sign forces  $-f_{i}^{(a)'}/(n-1)$  with zero net resultant at the centres of other connected elements. The corresponding surface traction is

$$\boldsymbol{q}_{ij}^{(a)\prime} = \begin{cases} f_i^{(a)\prime} / A_j^{(a)} & \text{if } i = j \\ -\frac{1}{n-1} f_i^{(a)\prime} / A_j^{(a)} & \text{if } i \neq j, \end{cases}$$
(A5)

where  $A_i^{(a)}$  is the area of element *j* connected to node '*a*'.

The total surface traction on an element is obtained by summarizing the contributions from the nodal force and moment of all of its neighbouring nodes.

Applying (2.24), the velocity at the element collocation point can be obtained. We relate the nodal velocity  $v^{(a)}$  and the velocity at the element collocation point  $v_i$  by

$$\boldsymbol{v}^{(a)} = \frac{1}{A^{(a)}} \sum_{i=1}^{n} \boldsymbol{v}_i A_i^{(a)}.$$
 (A 6)

The continuum-based shell element used here stems from the Reissner–Mindlin shell theory (see Belytschko *et al.* 2000). In this approach, the fibre is not necessary to be perpendicular to the shell reference surface, and it can rotate locally. The local fibre rotation is determined based on the nodal moments as follows.

Let  $q_{BE}^{\beta\nu}$  denote the global surface traction vector attributed to the nodal moments, we obtain the global velocity vector  $v_{BE}^{\beta\nu}$  at the element collocation point due to  $q_{BE}^{\beta\nu}$  as

$$\boldsymbol{v}_{\boldsymbol{B}\boldsymbol{E}}^{\boldsymbol{f}\boldsymbol{b}\prime} = (\boldsymbol{I} - \boldsymbol{D})^{-1} \boldsymbol{S} \boldsymbol{q}_{\boldsymbol{B}\boldsymbol{E}}^{\boldsymbol{f}\boldsymbol{b}\prime}.$$
 (A7)

If node '*a*' rotates locally as a rigid body with a local angular velocity  $\omega^{(a)}$ , then the velocity at the collocation point of connected elements can be written as

$$\boldsymbol{v}_{i}' - \boldsymbol{v}^{(a)\prime} = \boldsymbol{\omega}^{(a)} \times \boldsymbol{r}_{i} = \begin{bmatrix} 0 & -\omega_{3}^{(a)} & \omega_{2}^{(a)} \\ \omega_{3}^{(a)} & 0 & -\omega_{1}^{(a)} \\ -\omega_{2}^{(a)} & \omega_{1}^{(a)} & 0 \end{bmatrix} \cdot \boldsymbol{r}_{i}, \qquad (A8)$$

where

$$\boldsymbol{v}^{(a)\prime} = \frac{1}{A^{(a)}} \sum_{i=1}^{n} \boldsymbol{v}_{i}^{\prime} A_{i}^{(a)}.$$
 (A9)

There are 3n equations but 3 unknowns  $(\omega_1^{(a)}, \omega_2^{(a)}, \omega_3^{(a)})$ , the three components of the angular velocity vector at node 'a'). A least square method is used to solve these equations for  $\mathbf{\omega}^{(a)}$ .

After the nodal velocities and the local angular velocities are obtained, at each time step we need to update both the locations of the nodes and the orientations of the shell elements at the nodes (i.e. the 'fibre orientation' mentioned in Hughes & Liu 1981b). The nodal coordinates are updated using the explicit Euler method. Then the fibre direction of node 'a',  $\mathbf{x}'^{(a)}$  (a unit direction vector), is updated first by averaging the normals of connected elements based on the updated coordinates, then by applying the local rotation  $\mathbf{\omega}^{(a)}$  using Hughes–Winget's formula as

 $\boldsymbol{x}^{\prime(a)} = \boldsymbol{\Phi}^{(a)} \boldsymbol{x}^{\prime(a)},\tag{A 10}$ 

where

$$\Phi^{(a)} = (I - \frac{1}{2}\Omega^{(a)})^{-1}(I + \frac{1}{2}\Omega^{(a)}),$$
(A 11)

$$\mathbf{\Omega}^{(a)} = \begin{bmatrix} 0 & -\omega_3^{(a)} \Delta t & \omega_2^{(a)} \Delta t \\ \omega_3^{(a)} \Delta t & 0 & -\omega_1^{(a)} \Delta t \\ -\omega_2^{(a)} \Delta t & \omega_1^{(a)} \Delta t & 0 \end{bmatrix},$$
(A12)

where  $\Delta t$  is the time step. For the axisymmetric case, a similar coupling algorithm is developed to the above three-dimensional case.

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