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Dynamics of Erythrocytes in Oscillatory Shear Flows: Effects of S/V Ratio

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By combining a multiscale structural model of erythrocyte with a fluid-cell interaction model based on the boundary-integral method, we numerically investigate the dynamic response of erythrocytes in oscillatory shear flows (OSFs). The goal is to develop a novel experimental method to test the structural robustness of erythrocytes in transient mechanical loads with small time scales, conditions closely imitating the mechanical environment *in vivo*. Following the discovery of multiple response modes (wheeling, mode 1 tank treading, and mode 2 tank treading) under these conditions (Zhu & Asaro, 2019), we concentrate on deformation and stress inside RBC driven by OSF, especially shear deformation of the membrane and the skeleton-bilayer dissociation stress, parameters that are related to mechanically induced structural remodeling such as vesiculation. Effects related to changes in surface area-to-volume (S/V) ratio are considered. Our results show that with the variation of the S/V ratio there could be significant change in terms of the occurrence of response modes even if other parameters are kept unchanged. For example, by reducing the S/V ratio of the cell, an asymmetric mode featuring a mixture of the wheeling and mode 2 tank treading responses is discovered. This mode is found to be associated with large skeleton-bilayer dissociation stress so that its potential impact on OSF-driven vesiculation should not be overlooked. By systematically examining the dependencies of skeleton deformation and skeleton-bilayer dissociation stress upon S/V, this study is critical for the development of the OSF technique in applications such as diagnosis since cell conditions are often reflected in its geometric properties.

1 Introduction

Erythrocytes, also known as red blood cells or RBCs, play important physiological roles in the transportation of oxygen and carbon dioxide around the body. To perform these functions this type of cell is characterized by a molecular structure capable of sustaining large dynamic deformations while maintaining its structural robustness and physiological functionality. The basic architecture includes a composite membrane made of lipid and proteins surrounding the cytosol inside which hemoglobin resides.

The erythrocyte membrane is usually viewed as a two-layer structure. The outer layer is a fluid-like lipid bilayer with large areal stiffness, finite bending stiffness, and tiny shear stiffness. The inner layer, on the other hand, is a porous network consisting of various proteins. The basic repeating unit of this network is called a *junctional complex* (JC), which is a hexagon formed by a central piece of actin protofilament together with (up to) six spectrin dimers. This molecular architecture imparts finite areal and shear stiffnesses, as well as rather small bending stiffness. Therefore the bending stiffness of the membrane is determined mostly by the lipid bilayer, whereas its shear stiffness is attributed mostly to the protein skeleton.

A notable characteristic is that the connection between the protein skeleton and the lipid bilayer is far from rigid. In fact, these two are connected at isolated pinning points through skeleton-attached transmembrane proteins such as band 3 and glycophorin C^{1–4}. These proteins can drift laterally inside the lipid bilayer so that the connectivity between the skeleton and the bilayer is dynamic, allowing the two to have different local areal and shear deformations despite the fact that the overall surface area of the cell is conserved due to the incompressibility of the lipid bilayer. Moreover, the skeleton-bilayer linkage may fracture under large transverse mechanical loads (i.e. negative contact stress) so that the bilayer may be separated from the skeleton and eventually shed from the cell as vesicles, as demonstrated by micropipette aspiration^{5,6}. It is hypothesized that mechanically induced structural remodelings such as vesiculation also occur in circulation, where RBCs undergo severe deformations comparable to those in micropipettes under the combined effect of fluid forcing and boundary effects, especially when they go through narrow openings such as inter-endothelial slits in spleen^{7,8} or attach to endothelial cells via cell-to-cell adhesion⁹. Unlike micropipette aspiration, the loading conditions a RBC sustain *in vivo* are essentially dynamic. For example, according to observation the typical time scale of RBC transition through splenic slit is $\mathcal{O}(0.1)$ second. It is thus necessary to develop experimen-

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tal technology to study the effect of small-time-scale dynamic loads on the structural integrity and structural stability of red blood cells. A straightforward approach involves the development of artificial slits in microfluidic channels to imitate the inter-endothelial slits in spleen. When cells are forced through these slits it is possible to reproduce mechanical loads similar to those *in vivo*^{10–16}. However, in experiments these slits are usually much wider than their counterparts in spleen due to jamming or damaging issues in narrow slits^{12,17}. Moreover, during these experiments relatively small number of cells are examined¹⁸, which are insufficient for statistical studies that are critical for characterization.

A novel high-throughput method has been proposed to test the mechanics of RBCs in transient loading by using OSFs^{19,20}. By adjusting the amplitude and frequency of these flows it is possible to study the effects of unsteady forces with different strength and time scale. Experiments show that dynamic stresses induced by OSFs are sufficient to trigger structural remodeling inside the cell and lead to vesiculation¹⁹. It was demonstrated that this method has the potential of being applied as a convenient tool to test the structural robustness of the cell, especially the robustness of the lipid-skeleton connectivity, which is closely related to cell conditions. Towards this end, comprehensive knowledge about the dynamics of erythrocytes in OSFs is critical. There are, however, only a handful of investigations into this problem^{21–23}. These studies are either two dimensional or limited in parametric range (e.g. small number of cycles, low frequency, or low strength of shear flow). Besides, they do not consider the internal structure of the cell so that the mechanical stress inside the cell induced by OSF has not been studied.

Recently, systematic simulations have been conducted to investigate the dynamic response of RBCs in OSFs by using a multiscale fluid-cell interaction model²⁴. It was discovered that depending on the shear amplitude, frequency, and initial orientation of the cell there exists four distinguishable response modes: wheeling, mode 1 tank treading, mode 2 tank treading, and irregular. The transition from one mode to another was observed in many cases. Under certain conditions mode competition occurs, leading to chaotic behavior. This work was concentrated in the mapping of response modes at different peak capillary numbers (hereby defined as $C_a \equiv \eta_1 R \dot{\gamma}_0 / \mu_s$, where $\dot{\gamma}_0$ is the amplitude of the shear rate, η_1 is the viscosity of the fluid outside of the RBC, R is the radius a sphere with the same surface area as the RBC, and μ_s is the shear stiffness of the skeleton without deformation), viscosity ratio between the inside and outside fluids, and frequency of the flow. There is little study about the correlation between these response modes and internal deformation/stress inside the cell, especially the shear deformation of the skeleton and the dissociation stress between the lipid bilayer and the skeleton, which are key factors in mechanically induced structural

remodeling.

Another important factor that has not been considered is the dependence of cell response upon its mechanical and geometric properties. Depending on its age and conditions, the size, shape, and mechanical properties (e.g. shear stiffness of the membrane) of a red cell vary. For example, during *in vivo* aging both the surface area and the internal volume of a RBC decrease in a way so that the S/V ratio decreases as well and the cell shape becomes more spherical²⁵. The deformability of a red cell in quasi-static or dynamic loads is primarily determined by three factors: the shear stiffness of the membrane, the internal viscosity, and the S/V ratio. Among these factors, the membrane stiffness is included in the capillary number C_a . The viscosity of the internal fluid is included in the viscosity ratio. Both have been studied in the aforementioned numerical work²⁴. The effect of S/V ratio on the dynamics of RBC in OSF, on the other hand, has not been examined in any existing numerical or experimental work about RBCs in OSF.

On the other hand, there do exist strong evidences that S/V ratio plays a pivotal role in determining mechanics and dynamics of red cells. For example, it was numerically demonstrated that in comparison with the shear stiffness, the S/V ratio has a much more pronounced effect upon the transit time of RBC through slits¹⁵. Indeed, when S/V decreases the rigidity of the cell rises dramatically until the cell becomes almost undeformable as its shape approaches a sphere, since the lipid bilayer has very large areal stiffness. Other studies have reached similar conclusions²⁶. The effect of S/V ratio on the dynamics of RBCs in OSF is this an intriguing topic requiring comprehensive investigations.

In addition to the fundamental interest in mechanics of RBC under unsteady loading conditions, motivation of the current study also comes from the potential application of the OSF technique in diagnosis of red cells with molecular defects caused by diseases, aging, or storage. These defects disturb the structural stability inside the cell, especially the robustness of the skeleton-bilayer connectivity. It is expected that the vesiculation behavior of a RBC in OSF, e.g. the rate of vesiculation and properties of the vesicles themselves, reflects such molecular-level changes. On the other hand, we are aware that these changes may also affect the macroscale properties of a cell, especially its S/V ratio, which may influence its dynamics in OSF, and subsequently the vesiculation property. To avoid ambiguity in data interpretation, it is necessary to isolate these effects from effects of structural robustness.

We hereby apply a fluid-cell interaction model to simulate dynamics of RBCs with different values of S/V ratio in OSF. The model combines a multiscale structural model of the RBC with a Stokes-flow-based boundary-element model of the surrounding flow field. In the multiscale structural model the lipid bilayer and the protein skeleton are depicted separately so that it is convenient to determine the interaction force be-

tween them, especially the dissociation stress in the transverse direction which may trigger the fracture of skeleton-bilayer connection sites and lead to vesiculation. We will use this model to study the effect of S/V ratio on the occurrence of various response modes for RBCs in OSF. The characteristics of each mode in terms of skeleton deformation and skeleton-bilayer interaction stress will also be examined.

The rest of the paper is organized in the following manner. We first describe the physical problem, including the geometry of a single RBC as well as the surrounding flow field to be studied. This is followed by a brief depiction of the fluid-cell interaction model. The detailed development and validation of this model are included in previous publications. Numerical results, such as configuration of RBCs with different S/V ratios, dynamic characteristics of the response modes including cell deformation, shear deformation of the membrane, and skeleton-bilayer dissociation stress, are presented. Finally, conclusions are drawn.

2 Physical problem

As shown in Fig. 1, we consider a RBC in a time-varying shear flow U . In a rectangular coordinate system (x, y, z) , the flow is in the x direction and $U = U(y, t) = \dot{\gamma}(t)y$. The shear rate $\dot{\gamma}(t) = \dot{\gamma}_0 \sin \omega t$ ($\omega = 2\pi/T$ is the frequency of the oscillatory shear flow, where T is the period of oscillation).

We start from a flaccid and unswollen cell with a biconcave shape described as²⁷

$$y = \pm 0.5R_0 \sqrt{1 - \frac{x^2 + z^2}{R_0^2}} \left[C_0 + C_1 \frac{x^2 + z^2}{R_0^2} + C_2 \left(\frac{x^2 + z^2}{R_0^2} \right)^2 \right], \quad (1)$$

where $C_0 = 0.21$, $C_1 = 2.03$, $C_2 = -1.12$, and $R_0 = 3.9 \mu\text{m}$.

The rotations of the cell around the x , y , and z axes are denoted as θ , ϕ , and ψ , corresponding to roll, yaw, and pitch motions. The initial orientation is represented by the initial values of these three angles, θ_0 , ϕ_0 , and ψ_0 . Among them θ_0 plays a significant role in determining the responses of RBCs in steady or unsteady shear flows^{24,28}. It is thus a key parameter in our simulations. The other two initial orientation angles, ϕ_0 and ψ_0 , are all set to be zero.

In addition to θ_0 and the oscillatory period T , the other important physical parameters are the viscosity ratio Λ and the peak capillary number Ca . Hereby Λ refers to the ratio between the fluid viscosities inside and outside of the cell, i.e. $\Lambda \equiv \eta_2/\eta_1$, where η_1 is the viscosity of the fluid outside of the RBC and η_2 is the viscosity of the internal fluid (chosen as $0.006 \text{ pN s } \mu\text{m}^{-2,29}$).

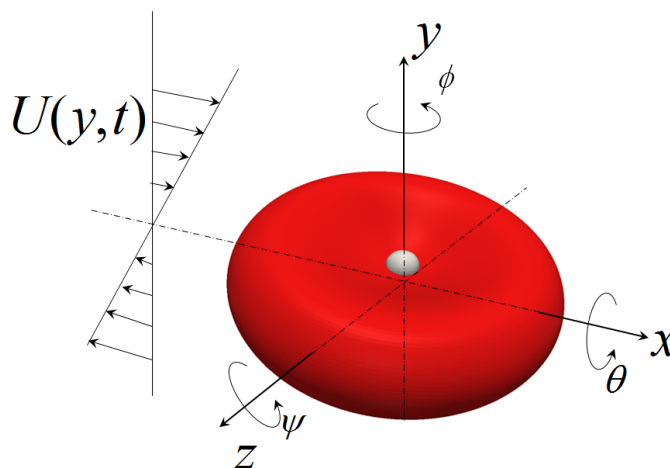


Fig. 1 Definition of the physical problem. The bead denotes the location of a dimple element.

3 Hierarchical fluid-cell interaction model

The simulations in this study will be conducted by using a multiscale fluid-cell interaction model. Our structural model includes three component models at different scales: in the complete cell level (Level III) the membrane is modeled as two layers of continuum shells using the finite element method – one representing the lipid bilayer and the other the skeleton^{6,30,31}; the constitutive properties of the inner layer (the skeleton) are obtained from a three-dimensional molecular-detailed model of the junctional complex (Level II)^{16,32}; the mechanical properties of spectrin (the protein that determines the deformability of the skeleton), including its folding/unfolding reactions, are obtained with a stress-strain model based on Arrhenius equation (Level I)^{16,33}.

In the literature, the dynamics of fluids inside and outside of RBCs are solved either *via* boundary-integral equations under the Stokes-flow framework^{34–37}, or through direct simulations of the Navier-Stokes equations using an immersed-boundary approach³⁸. Computationally, the boundary-integral method is more efficient since it only discretizes the solid-fluid interfaces (hereby the cell surface and other solid boundaries that may exist). It is thus employed in our model.

In the low Reynolds number regime, we employ the Stokes/Oseen flow theory and mathematically describe the flow fields inside and outside of the cell by using the following

boundary integral equation³⁹

$$\begin{aligned} \mathbf{v}(\mathbf{x}_0, t) &= \frac{2}{1+\Lambda} U(\mathbf{x}_0, t) \\ &- \frac{1}{4\pi\eta_1(\Lambda+1)} \iint_{\Gamma_c} \mathbb{G}(\mathbf{x}, \mathbf{x}_0) \cdot \Delta \mathbf{t}(\mathbf{x}, t) d\Gamma(\mathbf{x}) \\ &+ \frac{1-\Lambda}{4\pi(1+\Lambda)} \iint_{\Gamma_c} \mathbf{v}(\mathbf{x}, t) \cdot \mathbb{T}(\mathbf{x}, \mathbf{x}_0) \cdot \mathbf{n}(\mathbf{x}) d\Gamma(\mathbf{x}), \end{aligned} \quad (2)$$

The equation above is satisfied at an arbitrary point on the cell surface $\mathbf{x}_0$ ($\mathbf{x}_0 \in \Gamma_c$, where Γ_c stands for the cell surface). $\iint$ denotes the principal value integration. $\mathbf{v}$ is the velocity. U is the prescribed background flow velocity. The matrices $\mathbb{G}$ and $\mathbb{T}$ contain the Green's function for velocity $\mathcal{G}_{ij}$ and the Green's function for stress $\mathcal{T}_{ijk}$, respectively. To elaborate,

$$\mathcal{G}_{ij}(\mathbf{x}, \mathbf{x}_0) = \frac{\delta_{ij}}{|\mathbf{x} - \mathbf{x}_0|} + \frac{(x_i - x_{0i})(x_j - x_{0j})}{|\mathbf{x} - \mathbf{x}_0|^3}, \quad (3)$$

and

$$\mathcal{T}_{ijk}(\mathbf{x}, \mathbf{x}_0) = -6 \frac{(x_i - x_{0i})(x_j - x_{0j})(x_k - x_{0k})}{|\mathbf{x} - \mathbf{x}_0|^5}, \quad (4)$$

where δ_{ij} is Kronecker's delta. $i, j = 1, 2, 3$. $x_1 = x, x_2 = y, x_3 = z$.

We have developed a boundary-element algorithm to solve Eq. (2)³⁰. It is then coupled with a finite-element model which solves the complete-cell response (Level III) by representing both the inner layer (the protein skeleton) and the outer layer (the lipid bilayer) as aggregations of shell elements. The fluid-structure coupling is achieved by enforcing that the fluid and the cell have the same velocity and balanced traction on their interface⁴⁰.

Extensive tests have been conducted, which have proven the validity and accuracy of this approach^{6,30,31}. Such tests are thus not included in this paper.

4 Results

For consistency, the physical properties of the RBC membrane, e.g. bending stiffness of the lipid bilayer, mechanical properties such as persistence length of the spectrin, and friction coefficient between the lipid bilayer, remain the same as those used in previous studies²⁴.

In the following simulations, the period T of the OSF is chosen to be within the range of 0.02s~0.1s. This choice is based on the following facts: a) according to *in vivo* observations the time scale of RBCs passing through splenic slits is around 0.1s⁴¹; b) our experiments show that higher frequency OSFs (e.g. around 50Hz) tend to generate large number of vesicles,

suggesting that they induce large cell deformations, and subsequently large internal stress inside a cell. The range of the peak capillary number C_a is between 1.03 and 3.09 – below that range the cell deformation is too small, whereas above this range the cell response is often irregular, a response mode one would like to avoid since the results are less definitive. The viscosity ratio Λ is set to be 1, i.e. the viscosities of the fluids inside and outside of the cell are the same. This viscosity ratio enables fast simulations since no iteration is needed to solve Eq. (2)³⁰. Other values of the viscosity ratio have been explored in previous studies and the effect of this parameter has been illustrated²⁴. In experiments the value of Λ can be adjusted by manipulating the viscosity of the surrounding fluid⁴².

Numerically, we employ 2000 evenly distributed finite elements on the lipid bilayer and the skeleton. Correspondingly, 1000 boundary elements are used to calculate fluid forcing on the cell via the low-Reynolds number boundary integral equation (Eq. (2)). The time step is chosen to be 1 μ s.

4.1 Resting configurations of cells with different S/V

To obtain the resting configuration of a RBC, we start from the biconcave state described in Eq. (1), whereby the surface area of the cell is $S_0 = 133\mu m^2$ and the volume $V_0 = 93\mu m^3$. Without any external load, the surface area is kept unchanged while the internal volume is changed gradually towards a target value of αV_0 . After reaching the target value, the simulation continues until a steady state is reached. In the new resting configuration determined this way the S/V ratio is changed by a factor of $1/\alpha$. The range of parameter α we consider is [0.9, 1.5]. This range includes almost all the possible values of α – above it the cell reaches a sphere, below it the cell collapses with topological changes to its shape. Typical resting shapes with different values of α are shown in Fig. 2.

The advantage of this method to change the S/V ratio is that with fixed surface area the parameter R in the definition of the peak capillary number C_a remains unchanged. As we fix the outside fluid viscosity η_1 and the shear stiffness of the cell μ_s there will be an one-to-one correlation between the peak capillary number and the amplitude of the shear rate $\dot{\gamma}_0$. This greatly simplifies the definition of the physical problem and allows effects of the parameters C_a and α to be studied separately.

In the literature the S/V ratio of erythrocytes is often represented by a dimensionless parameter named the *sphericity index*, which is defined as $4.84V^{2/3}/S$. As the value of this parameter increases towards one the shape of the cell approaches a sphere. As shown in Table 1, the range of α considered in this work corresponds to a range of the sphericity index between 0.7 and 0.98. According to measurements, in normal population of human erythrocytes, the sphericity index distributes between 0.71 and 0.86⁴³. Another study shows that

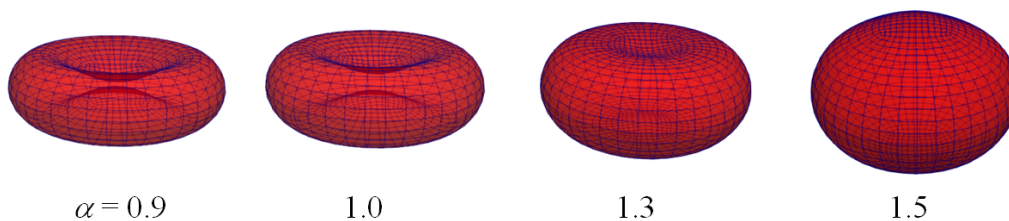


Fig. 2 Resting shapes of the cell with different values of the volume factor α .

during the aging process of RBC there is a 2.4% decrease in volume but a 18% decrease in surface area so that the sphericity index of old cells rises to around 0.9 if the original value is 0.75²⁵. The value of the sphericity index can be much larger in diseased cells. For instance, in cells affected by hereditary spherocytosis the shape approaches a sphere⁴⁴. These data demonstrate that the range of α (or sphericity index) chosen in this study is relevant.

Table 1 Relation between α and the sphericity index.

α	0.9	1.0	1.1	1.2	1.3	1.4	1.5
sphericity index	0.7	0.75	0.8	0.84	0.89	0.94	0.98

4.2 Characteristics of response modes

The response of the cell is characterized by the following quantities:

1. Trajectories of the dimple elements (i.e. elements originated from the dimples of the cell in the resting state). We define two such elements, A and B, from the two dimples. These elements are depicted as beads in the figures. We will then record the z -positions of these two elements, z_A and z_B . Since the centroid of the cell remains at the origin, z_A and z_B measure the drift of elements A and B from the central plane $z = 0$, which can be used to distinguish the response modes: in mode 1 tank treading they are close to zero; in wheeling they are small but finite ($|z_A|, |z_B| \sim 0.5 - 1 \mu\text{m}$ for unswollen cells); in mode 2 tank treading A and B drift away from the central plane so that $|z_A|$ and $|z_B|$ are relatively large ($\sim 2 - 3 \mu\text{m}$).
2. The maximum shear deformation of the skeleton. The in-plane deformations of the skeleton are represented by the areal and shear deformations. The areal deformation is very small in our scenario, so that the shear deformation is the primary parameter. In our study the shear deformation is defined as λ_1/λ_2 , where λ_1 and λ_2 are the two principal stretches ($\lambda_1 \geq \lambda_2$). At any instant, the maximum value of λ_1/λ_2 over the whole membrane is defined

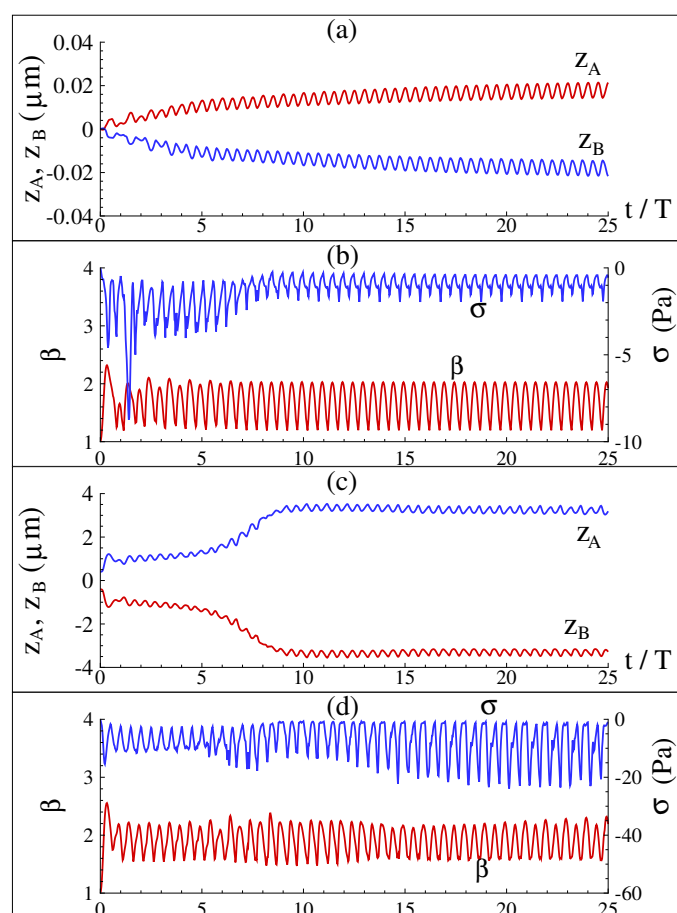


Fig. 3 Time histories of z_A and z_B (the z -locations of the two membrane elements originated from the dimples), the maximum shear deformation β , and the maximum dissociation stress σ . (a) and (b) are from a case with $\theta_0 = 0$. (c) and (d) are from another case with $\theta_0 = 90^\circ$. $T = 0.04\text{s}$, $C_a = 2.06$, $\alpha = 1$.

as the maximum shear deformation β . The peak value of β over a complete oscillatory cycle is defined as the peak shear deformation β_p .

3. The maximum dissociation stress. The interaction between the lipid bilayer and the protein skeleton includes two parts, friction in the in-plane direction and contact stress in the out-of-the-plane direction. The negative value of the contact stress corresponds to a tendency of dissociation, which is thus termed the *dissociation stress*. The maximum value (in terms of the absolute value) of this dissociation stress over the whole membrane is defined as σ . The peak value of σ (also in terms of the absolute value) over a complete cycle is the peak dissociation stress σ_p .

Among these, the shear deformation of the skeleton and the dissociation stress have not been considered systematically in the previous work²⁴.

We start by concentrating on a case with $T = 0.04$ s, $C_a = 2.06$, and $\alpha = 1$, which is representative since it displays three dynamic modes, including mode 1 tank treading, mode 2 tank treading, and wheeling responses depending on θ_0 and the duration of OSF.

In Fig. 3 we plot the time histories of z_A , z_B , β and σ at $\theta_0 = 0$ and $\theta_0 = 90^\circ$. It is clearly seen that the initial orientation plays a pivotal role in determining the dynamic response of a cell as well as the deformation and internal stress inside it. Specifically, the mode 1 tank treading response occurs when $\theta_0 = 0$. On the other hand, when $\theta_0 = 90^\circ$ the cell starts with the wheeling mode and gradually switches to the mode 2 tank treading response after a number of cycles.

In terms of shear deformation in the cell membrane, there is little difference among the three response modes – in all cases the peak value β_p is around 2. However, the dissociation stresses reached in these modes differ significantly from each other. For example, the peak dissociation stress σ_p in mode 1 tank treading is only around -1.5 Pa. The corresponding values in wheeling and mode 2 tank treading are -10 Pa and -16 Pa, respectively.

The cell deformations and distributions of shear deformation and bilayer-skeleton contact stress in the aforementioned three response modes are displayed in Figs. 4 to 6. It is seen that in the two tank treading modes the peak shear stress occurs in the membrane far from the central plane ($z = 0$) at the moment of maximal stretching. In the wheeling mode the peak shear occurs in the (now compressed) dimple region, near the moment of fully stretching as well.

The occurrence of large contact stress, on the other hand, is usually associated with large membrane curvature⁶. This is clearly shown in Fig. 5 and Fig. 6. In the former case, large membrane bending occurs near the location where the dimple is compressed, together with significant membrane shearing

nearby large contact stress is created. In the latter case, the high-frequency back-and-forth motion creates a phenomenon similar to the ‘infecting’ deformation when a cell squeezes through a narrow slit^{13,16,45}. When this happens the membrane bends inward to form a concave area akin to the dimples. This creates large membrane bending, and subsequently, large contact stress (including dissociation stress) between the bilayer and the skeleton.

We also notice that during the transient motion in the starting process (see Fig. 3b when $t < 7T$) or the transition from one mode to another (see Fig. 3d when $6T < t < 8T$) large dissociation stress may occur – in some cases it could be several times larger than the steady-state value, as demonstrated in Fig. 3b. However, this transient effect usually lasts for just a few cycles.

The aforementioned response modes, i.e. wheeling, mode 1 tank treading, and mode 2 tank treading, have all been reported in²⁴. Another response mode, the irregular mode, occurs mostly in cases with high capillary numbers. These cases are circumvented in the current study by limiting the range of capillary number since this particular mode brings uncertainty and unpredictability. On the other hand, it is found that at certain values of the S/V ratio, there exists an additional response mode, hereafter referred to as the asymmetric mode. As shown in Fig. 7, in this mode the cell shape is no longer symmetric with respect to the $z = 0$ plane. Instead, it is essentially a mixture of the wheeling motion and the mode 2 tanking treading motion, one on each side of the $z = 0$ plane. At the wheeling side there is significant local bending, causing relatively large dissociation stress between the skeleton and the bilayer as demonstrated in the inset of Fig. 7.

4.3 Occurrence of response modes at various S/V ratios

In Fig. 8 we plot the response modes near the beginning and end of the simulation at different values of swollen factor α and period T when the initial orientation $\theta_0 = 90^\circ$. The duration of our simulations is chosen to be $50T$, which is close to what was used in experiments¹⁹.

It is seen that at the beginning of the simulation (Fig. 8a,c,d) there are mostly two dynamic modes, wheeling and mode 2 tank treading. The wheeling mode occurs when T and/or α are small, i.e. the lower/left part of the $\alpha - T$ space. The mode 2 tank treading response, on the other other dominates the upper/right part of the $\alpha - T$ space, a region that enlarges with the increase of the peak capillary number C_a . The asymmetric mode occurs within very narrow ranges of α and T . We note that no results are available at certain combinations of α and T when $C_a = 3.09$, e.g. when $\alpha = 0.9$ and $T = 0.08$ s. These are cases when different parts of the inner surface of the membrane touch each other during its deformation so that the boundary-integral problem becomes singular.

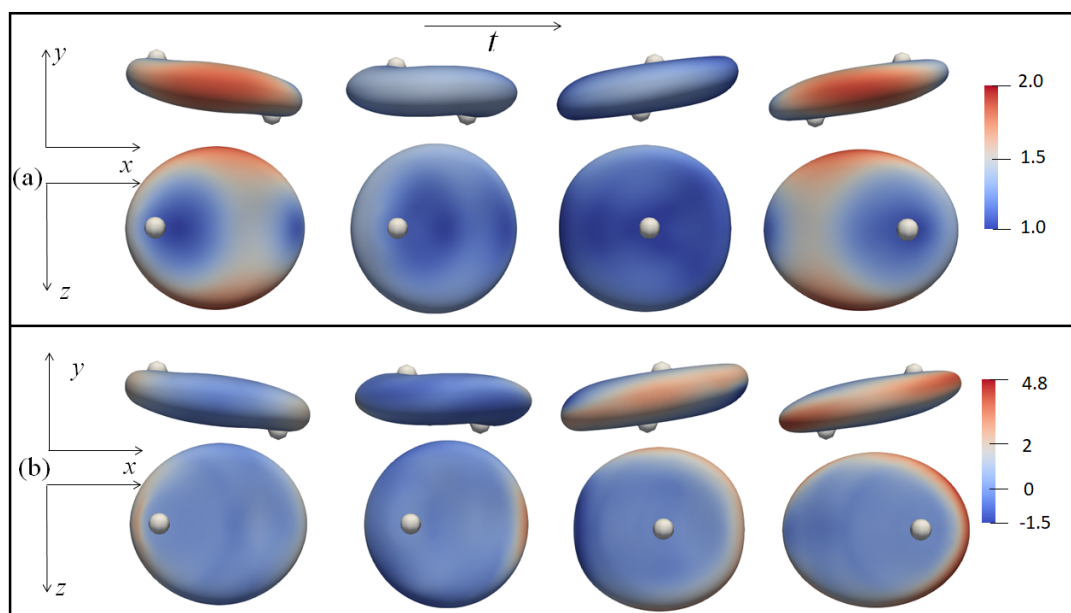


Fig. 4 (a) Shear deformation (λ_1/λ_2) of the membrane and (b) contact stress between the skeleton and the lipid bilayer (in Pascal) during a mode 1 tank treading response between $t = 8T$ and $t = 8.375T$. The beads represent positions of membrane elements originated from the dimple areas. $T = 0.04s$, $\theta_0 = 0^\circ$. $\alpha = 1$, $C_a = 2.06$.

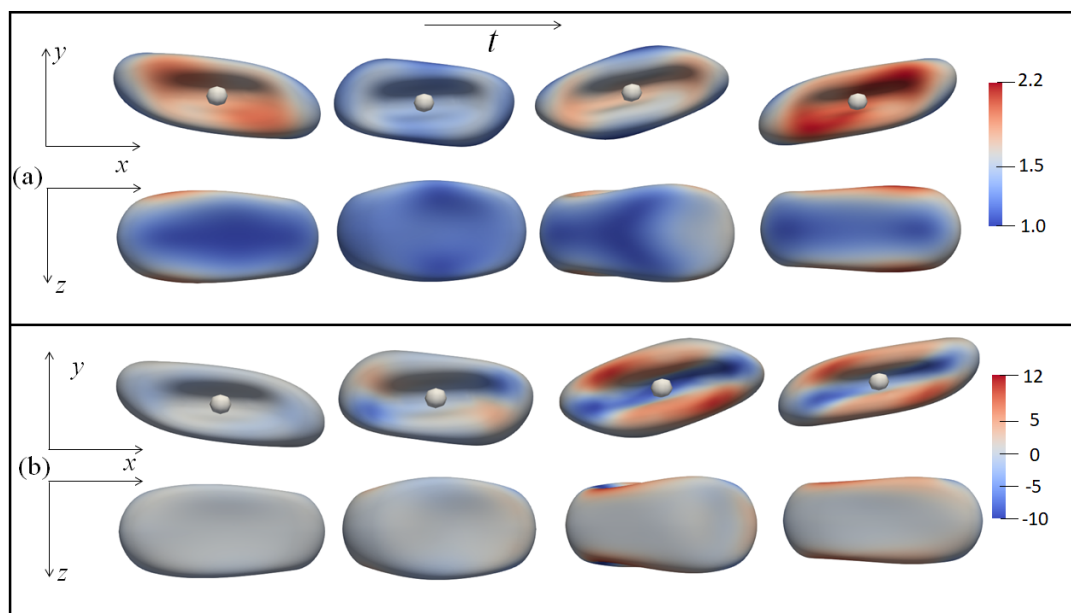


Fig. 5 (a) Shear deformation (λ_1/λ_2) of the membrane and (b) contact stress between the skeleton and the lipid bilayer (in Pascal) during a wheeling response between $t = 2T$ and $t = 2.375T$. The beads represent positions of membrane elements originated from the dimple areas. $T = 0.04s$, $C_a = 2.06$, $\alpha = 1$, $\theta_0 = 90^\circ$.

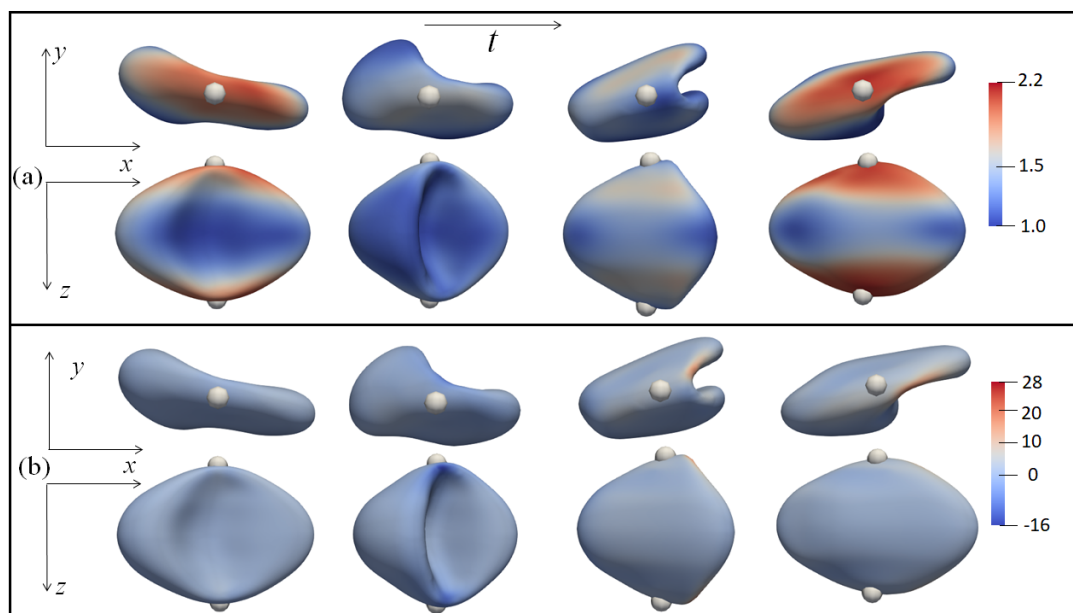


Fig. 6 (a) Shear deformation (λ_1/λ_2) of the membrane and (b) contact stress between the skeleton and the lipid bilayer (in Pascal) during a mode 2 tank treading response between $t = 15T$ and $t = 15.375T$. The beads represent positions of membrane elements originated from the dimple areas. $T = 0.04\text{s}$, $C_a = 2.06$, $\alpha = 1$, $\theta_0 = 90^\circ$.

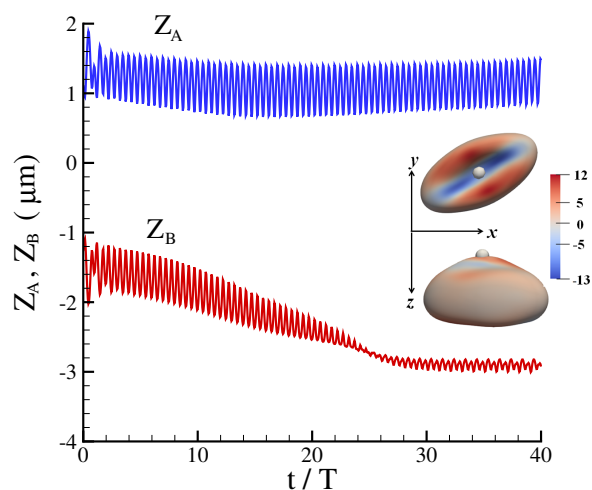


Fig. 7 Occurrence of the asymmetric mode with the swollen cell at $\alpha = 1.25$ shown by the time history of the z locations of the dimple elements. The inset displays the side view and the top view of the cell shape at $t = 30.3T$. The contour shows the contact stress (in Pascal) between the lipid bilayer and the skeleton. The beads represent positions of membrane elements originated from the dimple areas. $T = 0.02\text{s}$, $C_a = 2.06$. $\theta_0 = 90^\circ$.

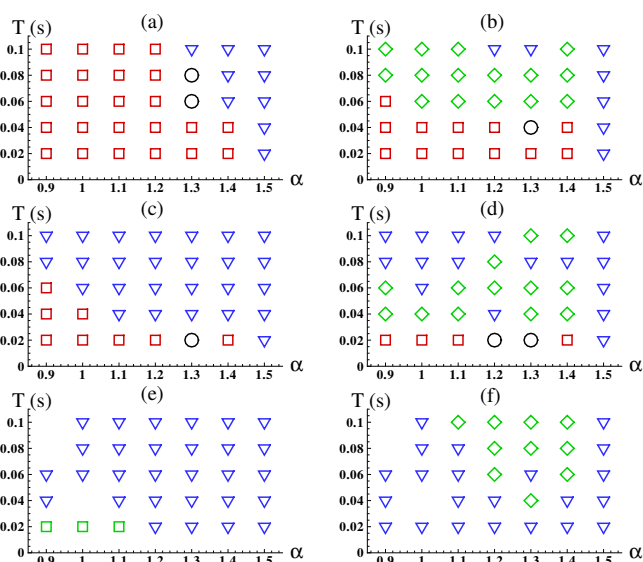


Fig. 8 Response modes at the beginning (a,c,e) and the end of simulation (b,d,f) with different values of swollen factor α at various oscillation period T . $\theta_0 = 90^\circ$. The values of the peak capillary number C_a are 1.03 (a,b), 2.06 (c,d), and 3.09 (e,f). The symbols represent wheeling ($\square$), mode 1 tank treading ($\diamond$), mode 2 tank treading (∇), and asymmetric mode ($\circ$).

Near the end of the simulation (Fig. 8b,d,f) there are mode switching events in many of the cases. Specifically, we find switching from wheeling to mode 1 tank treading (e.g. at $C_a = 1.03$, $T = 0.08$ s and $\alpha = 1$), from wheeling to mode 2 tank treading (e.g. at $C_a = 3.09$, $T = 0.02$ s and $\alpha = 1.1$), from wheeling to asymmetric response (e.g. at $C_a = 2.06$, $T = 0.02$ s and $\alpha = 1.2$), from mode 2 tank treading to mode 1 tank treading (e.g. at $C_a = 2.06$, $T = 0.1$ s and $\alpha = 1.3$), and from asymmetric response to mode 1 tank treading (e.g. at $C_a = 1.03$, $T = 0.06$ s and $\alpha = 1.3$). As a consequence of these transitions all the four response modes are observed. However, we need to point out that the responses shown in these three sub-figures are not necessarily the final state of response. Due to the finite simulation time additional transitions may occur after $t = 50T$. Hereby these events are not considered.

In general, at the beginning of the simulation the increase in α encourages the mode 2 tank treading mode instead of the wheeling mode. Geometrically this is easy to understand since the amount of deformation that is required from a swollen cell to the mode 2 configuration is smaller than the one with a flaccid cell as the starting point. The situation at the end of simulation is more complicated due to the unpredictability in the occurrence of mode switching. A notable feature is that in cases with small T ($T = 0.02$ s) and large C_a ($C_a = 3.09$) the occurrence of different response modes is less sensitive to α . The implication is that in these cases there is more certainty about the response mode even if there is uncertainty about the exact S/V ratio of the cell itself.

We have also conducted systematic simulations with $\theta_0 = 0^\circ$ within the same ranges of C_a , T , and α . Under these conditions in most of the cases the response remains as mode 1 tank treading. The only exception happens with $T = 0.1$ s, where there could be switching towards mode 2 tank treading if C_a is larger than 2.06.

For other values of θ_0 , the cell undergoes one of the aforementioned response modes or more than one modes in succession of each other depending on how close θ_0 is to 90° . For instance, systematic simulations have been conducted by fixing $T = 0.04$ s, $\alpha = 1$, $C_a = 2.06$, and varying θ_0 gradually from 0° to 90° . The result show that when $\theta_0 > 83^\circ$, the response is similar to the one with $\theta_0 = 90^\circ$, which includes wheeling and mode 2 tank treading. Otherwise the response is similar to the one with $\theta_0 = 90^\circ$, i.e. mode 1 tank treading. The implication is that in comparison with mode 1 tank treading, wheeling and mode 2 tank treading responses may be relatively rare events.

4.4 Effects of S/V on β_p and σ_p

The occurrence of mechanically induced structural remodeling in a RBC is closely related to the distribution of deformation and mechanical loads in it, especially the shear deformation

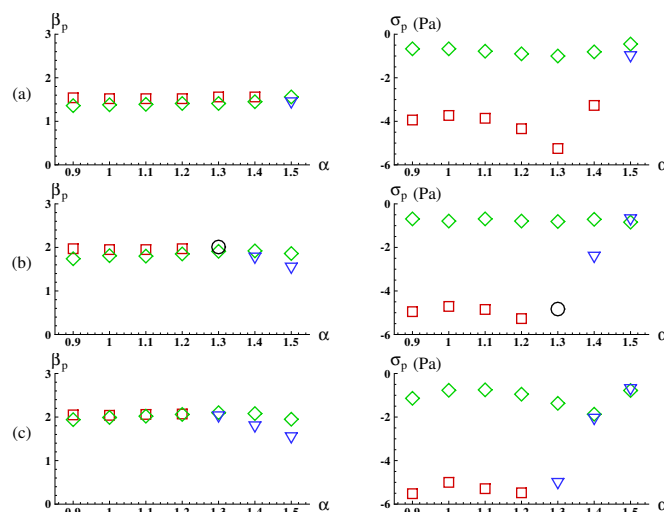


Fig. 9 The peak shear deformation β_p and peak dissociation stress σ_p (in Pascal) with different values of swollen factor α at (a) $T = 0.02$ s, (b) $T = 0.06$ s, and (c) $T = 0.10$ s. $C_a = 1.03$. The symbols represent wheeling ($\square$), mode 1 tank treading ($\diamond$), mode 2 tank treading (∇), and asymmetric mode ($\circ$).

tion of the skeleton (which may trigger dissociation between neighboring JCs) and the contact stress between skeleton and lipid bilayer (which may cause fracture of the skeleton-bilayer linkage). In our study we use two parameters to depict these effects, the peak shear deformation β_p and the peak dissociation stress σ_p .

In Fig. 9 we plot the values of β_p and σ_p at different combinations of T and α when $C_a = 1.03$. At each set of T and α there may exist multiple response modes depending on the initial orientation of the cell and the (possible) occurrence of mode switching, leading to multiple values of β_p and σ_p . These values are distinguished by using different symbols for different response modes. According to the results, the values of both β_p and σ_p remain more or less steady within the range $0.9 < \alpha < 1.3$. Beyond that range we observe a moderate drop of β_p and a dramatic decrease in σ_p (in terms of its absolute value). This is explained by the fact that when $\alpha > 1.3$ the cell shape approaches a sphere (see Fig. 2) so that its deformability is greatly compromised. Consequently both the shear and bending deformations of the cell membrane decrease.

Figure 10 displays the dependencies of β_p and σ_p upon T and α at the intermediate capillary number of $C_a = 2.06$. There are several notable characteristics in these results: 1) the existence of unusually high dissociation stress at $T = 0.02$ s and $\alpha \sim 1.2$, which is attributed to the occurrence of the asymmetric mode. 2) With the increase in T the drop in σ_p occurs at lower values of α . For example, when $T = 0.06$ s it happens after $\alpha > 1.2$, whereas when $T = 0.10$ s the drop is observed after $\alpha > 1.1$. 3) Another abnormality is seen at $T = 0.10$ s

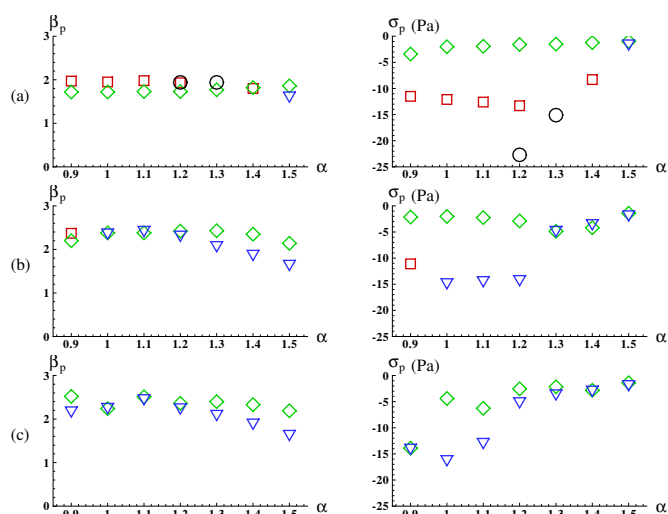


Fig. 10 Same as Fig. 9 except that $C_a = 2.06$.

and $\alpha = 0.9$, where the mode 1 tank treading response leads to a σ_p comparable to the one during the mode 2 tank treading response (both are around -14 Pa). This appears to contradict the observation in §4.2 that mode 1 is usually associated with small values of σ_p due to smaller cell bending in comparison with the other modes. For explanation in Fig. 11 we plot the cell deformation in this case over half an oscillatory period. It is clearly seen that due to the enhanced deformability of the cell with increased S/V (in this case by about 10%), local infolding happens, which is very similar to the infolding scenario in mode 2 tank treading response (see Fig. 6). This mechanism causes large dissociation stress.

At the high capillary number of $C_a = 3.09$, the behavior is similar to the one at $C_a = 2.06$ as shown in Fig. 12. In all the cases shown in the figure the values of β_p and σ_p do not change much between $\alpha = 0.9$ and $\alpha = 1.1$. Above that range both β_p and σ_p decrease (especially σ_p), even if there is no change in terms of the response modes in, for example, the case when $T = 0.10$ s.

5 Conclusions and discussion

Motivated by the potential usage of OSF-induced erythrocyte vesiculation in applications such as assessment of cell conditions and diagnosis of diseases, we conducted numerical study by using a fluid-cell interaction model to examine cell motion, deformation, and internal stress at different OSF conditions. Our focus in this work is the distribution of internal cell deformation and stress that may trigger structural remodeling (e.g. skeleton-bilayer dissociation) that leads to vesiculation. We first examined the shear deformation and dissociation stress in different dynamic modes, wheeling, mode 1 tank treading,

and mode 2 tank treading. There is no big difference in terms of the shear deformation yet the wheeling and mode 2 tank treading modes induce much larger dissociation stress than the mode 1 tank treading mode does.

A primary focus of this study is the influence of changes in geometrical properties of the cell. Specifically we studied cases with different S/V ratios. Towards that end we considered cases in which the surface area of the cell is fixed while its internal volume increased. The effects of this change are characterised in three aspects: 1) the effect on the occurrence of response modes, 2) the effect on the peak shear deformation β_p of the skeleton, and 3) the effect on the peak dissociation stress σ_p between lipid bilayer and skeleton.

Our results indicate that changes in the S/V ratio do affect the occurrence of response modes – the cell may undergo different response modes at different values of S/V ratio even if other parameters are not changed. In certain cases with swollen cells a new asymmetric mode has been observed. This mode leads to large values of σ_p . This geometric parameter also affects β_p and σ_p . In general a reduction of this ratio leads to decreases in both β_p and σ_p . The effect may be subtle when the change in S/V is small or moderate. However, the effect becomes dramatic after the ratio decreases below a critical value which depends on the peak capillary number C_a and the oscillatory period T .

Our results suggest that to apply the OSF technique as a tool to study alternations in the stability of the molecular architecture caused by defects or diseases, it is necessary to carefully choose the OSF parameters to avoid conditions in which changes in the geometrical properties of the cell dramatically affect its dynamics and internal stress, and make sure that the effect of these factors on the vesiculation behavior is relatively small. Otherwise the influence of S/V must be taken into account while interpreting the results.

On the other hand, it is noteworthy that changes in α (or the sphericity index) is often associated with variations in other important parameters, e.g. the surface area, the shear stiffness, and the viscosity of the internal fluid. Among these the surface area and the shear stiffness are both included in the definition of the capillary number C_a . The viscosity of the internal fluid affects the viscosity ratio. For complete knowledge about this problem a comprehensive parametric investigation of the effects of the capillary number, the viscosity ratio, the sphericity index, and other parameters (e.g. the viscosity of the membrane) is needed.

Studies like this may lead to novel methods in ektacytometry. For example, by combining the OSF technology with the Osmoscan and Lorrca methods it is possible to develop new techniques to measure not only the deformability of red cells but also robustness of their internal molecular architecture. During Osmoscan tests the S/V ratio of a cell is significantly changed so that knowledge about the effect of this

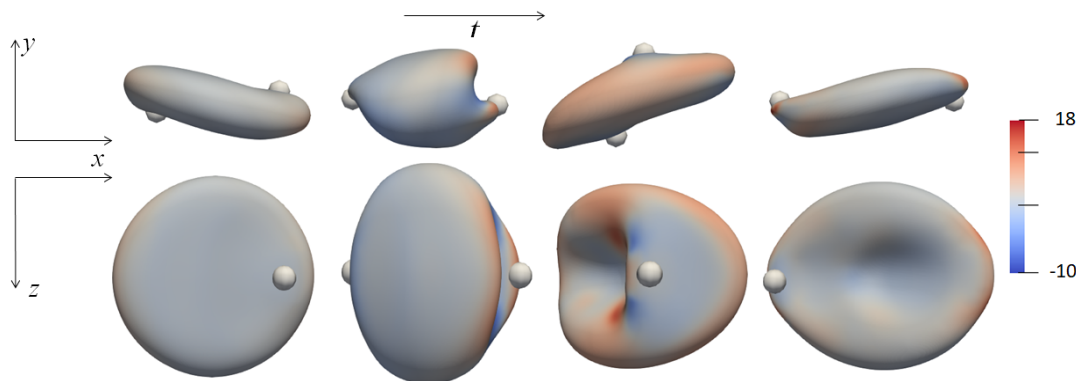


Fig. 11 Cell deformation in a mode 1 tank treading response between $t = 7T$ and $t = 7.375T$. The contour shows the contact stress between the skeleton and the lipid bilayer (in Pascal). The beads represent positions of membrane elements originated from the dimple areas. $C_a = 2.06$, $T = 0.10s$, $\theta_0 = 0^\circ$, $\alpha = 0.9$.

parameter is critical.

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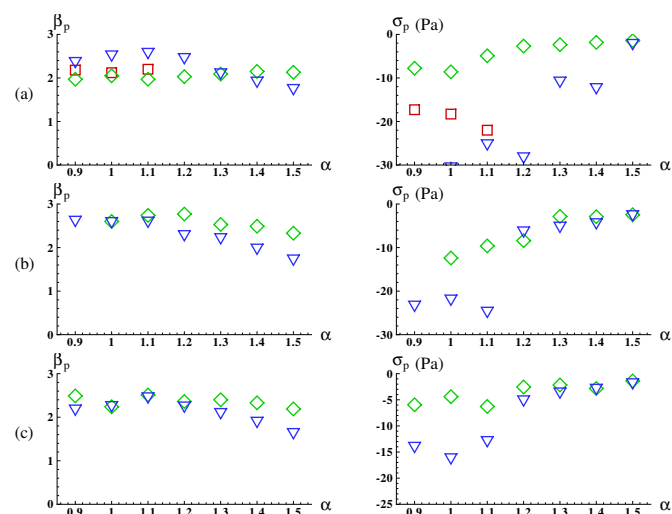


Fig. 12 Same as Fig. 9 except that $C_a = 3.09$.

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