Supplementary materials

1. Reference states
A subdivision method is used to generate the triangular mesh of reference states. Starting from an icosahedron with 20 regular triangles and 12 vertices (see Figure 1a), these vertices are projected to the surface of a sphere with the same surface area as an RBC. For each edge, a new vertex is created at the middle of it, and each element is divided into four elements. These new vertices are then projected onto the same spherical surface along the radial direction, completing one iteration of subdivision as shown in Figure 1b. The above procedure is repeated until the desired mesh size is reached (see Figure 1c). Thus the reference state for $V_I/V_S = 1$ and $V_{II}/V_S = 1$ is obtained.

![Image](image-url)

**Figure 1** Mesh generated with subdivision method: (a) the icosahedron; (b) the mesh obtained after one iteration of subdivision; (c) the final mesh. The above meshes have 20, 80, 1280 triangular elements, respectively.

For non-spherical reference states, we first calculate the length of the semi-major axis as $a$. A mesh like Figure 1c is zooming into a spherical surface with radius $a$. Then all the vertices are projected to the surface of the reference states along the $y$-direction, while the $x$- and $z$-coordinates are fixed.

After triangulation, the curvature of each vertex is calculated as the spontaneous curvature $c_0$ of the RSL. The estimation of the curvature follows the study of Petitjean and is not repeated here.

2. Simulation method
For the membrane with zero thickness and negligible inertial effect, the momentum equation can be written as

$$\mu \dot{v} = f^{int} + f^{ext},$$

where $\mu$ is the viscous damping coefficient, $v$ is the membrane velocity, and $f^{int}$ and $f^{ext}$ are the membrane force and external force. Let $\Gamma$ be the calculation domain of the membrane. Using the principle of virtual work, the variation form of equation (1) is given as

$$\int_\Gamma \delta v \cdot \mu \dot{v} d\Gamma = \int_\Gamma \delta v \cdot f^{int} d\Gamma + \int_\Gamma \delta v \cdot f^{ext} d\Gamma,$$

where $\delta v$ is an arbitrary test function.

In the finite-element approximation, the calculation domain $\Gamma$ is divided into element domains $\Gamma_e$. As shown in section 1, triangular elements are used to discretize the membrane. The total number of nodes and elements are $n_n$ and $n_e$, respectively. Each element is
transferred to the $\xi\eta$ plane by rigid body translation and rotation, and its barycenter coincides with the origin (see Figure 2).

Figure 2 Schematic of the local coordinate system of a triangular element.

With the linear in-plane interpolation, any position $x$ of an element is given as

$$
x(\xi, \eta) = \sum_{i=1}^{3} N_i(\xi, \eta)x_i
$$

where $N_i$ is the shape function at node $i$ of the element, and $(\xi, \eta)$ refers to the local coordinate system. In present work, the shape functions are given as

$$
N_1 = \frac{\xi_2\eta_3 - \xi_3\eta_2 + (\eta_2 - \eta_3)\xi + (\xi_3 - \xi_2)\eta}{2S_e},
$$

$$
N_2 = \frac{\xi_3\eta_1 - \xi_1\eta_3 + (\eta_3 - \eta_1)\xi + (\xi_1 - \xi_3)\eta}{2S_e},
$$

$$
N_3 = \frac{\xi_1\eta_2 - \xi_2\eta_1 + (\eta_1 - \eta_2)\xi + (\xi_2 - \xi_1)\eta}{2S_e},
$$

where $S_e$ is the area of the element. Thus at each element, the Galerkin approximation is employed as

$$
\mathbf{v} = \mathbf{N}v_e, \delta \mathbf{v} = \mathbf{N}\delta v_e.
$$

The subscript ‘$e$’ represents element variables, ranging from 1 to $n_e$. $\mathbf{N} = (N_1, N_2, N_3)$ is the shape function vector. The element velocity matrix $\mathbf{v}_e = (v_1, v_2, v_3)^T$ assembles the velocity vectors of all the nodes within the element, and $\delta \mathbf{v}_e$ is the corresponding part of $\delta \mathbf{v}$. Using equation (5), equation (2) can be discretized as follow:

$$
\sum_{G} \int_{\Gamma_G} \delta \mathbf{v}_e^T \mathbf{N} \mu \mathbf{N} \mathbf{v}_e d\Gamma_G = \sum_{G} \int_{\Gamma_G} \delta \mathbf{v}_e^T \mathbf{N} \mathbf{f}_{int} d\Gamma_G + \sum_{G} \int_{\Gamma_G} \delta \mathbf{v}_e^T \mathbf{N} \mathbf{f}_{ext} d\Gamma_G,
$$

Since $\mathbf{v}_e$ and $\delta \mathbf{v}_e$ are $3 \times 3$ element matrices, they are related to the $n_n \times 3$ global matrices $\mathbf{v}_g$ and $\delta \mathbf{v}_g$ through transformation matrix $\mathbf{L}_e$ as

$$
\mathbf{v}_e = \mathbf{L}_e \mathbf{v}_g, \delta \mathbf{v}_e = \mathbf{L}_e \delta \mathbf{v}_g.
$$

Substituting equation (7) into equation (6) and eliminating $\delta \mathbf{v}_g$, we obtain

$$
\sum_{G} \left( \mathbf{L}_e^T \int_{\Gamma_G} \mathbf{N}^T \mu \mathbf{N} d\Gamma_G \mathbf{L}_e^T \right) \mathbf{v}_g = \sum_{G} \left( \mathbf{L}_e^T \int_{\Gamma_G} \mathbf{N}^T \mathbf{f}_{int} d\Gamma_G \right) + \sum_{G} \left( \mathbf{L}_e^T \int_{\Gamma_G} \mathbf{N}^T \mathbf{f}_{ext} d\Gamma_G \right).
$$

The above equation (8) can be written in matrix form as

$$
\mathbf{C} \mathbf{v}_g = \mathbf{f}_{g, \text{int}}^T + \mathbf{f}_{g, \text{ext}}^T,
$$

where $\mathbf{f}_{g, \text{int}}$ and $\mathbf{f}_{g, \text{ext}}$ are the global internal and external force matrices. The assembled damping matrix is
\[ C = \sum_{e} \left( L_e^T \int_{\Gamma_e} N^T \mu N d\Gamma_e L_e \right). \] (10)

For detailed description of the finite element method, please refer to the study of Zienkiewicz and Taylor\(^2\) and Peng\(^3\).

It should be noted that in equation (10), \( \mu \) is a parameter that only affects the time scale of the simulation. With larger value of \( \mu \), the whole shape recovery process would be longer, and vice versa. Thus in the present work, the dimensionless time is the major concern. By choosing the unit of \( \mu \) as N m\(^{-1}\), the time \( t \) obtained from equation (9) becomes dimensionless naturally.

3. Resting shapes
The resting shapes are obtained by the deflation process proposed by Lim et al.\(^4\) The deflation for case 1 is shown in Figure 3 as an example. The initial shape is the same as the RSC with \( V_I/V_s = 0.997 \) (see Figure 3a), and the RSL is characterised by the dimensionless spontaneous curvature \( c_0^* = 4 \). The volume of the initial shape is 144 \( \mu m^3 \), larger than the RBC volume as 94 \( \mu m^3 \). Due to the volume constraint force, the cell volume would be gradually reduced, which in turn deforms the membrane. The deformation of membrane is governed by

\[ Cv_g = f_g^{int}, \] (11)

where \( C \) is the assembled damping matrix, \( v_g \) is the assembled velocity vector and \( f_g^{int} \) is the total elastic force of the membrane. The advection of vertices is calculated as \( x^{(n+1)} = v_g \cdot dt + x^{(n)} \), where \( n \) refers to the nth iteration. After several dozen iterations, the cell becomes the shape as shown in Figure 3b, and its volume has already been reduced to 94 \( \mu m^3 \). The cell finally reaches an equilibrium state, which is the resting shape (see Figure 3c). The resting shapes for other cases are obtained through similar process.

![Figure 3 Deflation for case 1: (a) the initial shape; (b) the intermediate shape after several dozen iterations; (c) the resting shape.](image)

The resting shapes of the cell at different values of \( V_I/V_s \) and \( c_0^* \) is shown in Figure 4. It seems that with certain RSC, a shape transition from stomatocyte (a bowl shape) to a discocyte (a biconcave shape) is observed as the value of \( c_0^* \) increases beyond a threshold value. This threshold \( c_0^* \) value increases with \( V_I/V_s \), which is qualitatively consistent with the results of Peng et al\(^5\) and Tsubota et al\(^6\). When \( V_I/V_s \) is less than 0.95, the bowl shape disappears, in quantitative agreement with the study of Tsubota et al\(^6\). In the study of Peng et al\(^5\), the threshold \( c_0^* \) values are larger than present simulation results, since they used a
multiscale membrane model, different from the continuum membrane model in present study.

Another feature is that for some parameter set, the resting shape may be a stomatocyte (bowl-like shape) or a discocyte (biconcave shape), depending on the initial shape of deflation. An asymmetric initial shape may lead to a bowl-like resting shape. This deformation hysteresis is consistent with the study of Tsubota et al. It is also interesting to notice that for the cell with two different resting shape, the membrane energy is lower for the stomatocyte. Thus the stomatocyte is the globally stable shape, while the discocyte is the locally stable shape.

![Figure 4](image.png)

Figure 4 Resting shapes of the cell at different values of $V_I/V_s$ and $c_0^*$. $V_I/V_s = 0.65$ corresponds to the biconcave shape.

The resting shapes of the cell with non-spherical RSLs are shown in Figure 5. Just like the spherical RSL cases, different initial shapes may also lead to stomatocyte and discocyte for the same parameter set. However, the cell with biconcave RSL has only biconcave resting shape, consistent with the results of Sinha and Graham.

![Figure 5](image.png)

Figure 5 Resting shapes of the cell at different values of $V_I/V_s$ and $V_I/V_c$. The volume ratio of 0.65 corresponds to the biconcave shape.

4. Optical tweezer test

In the study of Siguenza et al., the shear modulus is set as $3.65 \times 10^{-6}$ N/m, higher than the shear modulus value ($2.5 \times 10^{-6}$ N/m) in present work. Their simulation results show discrepancy from the experiments for the transverse diameter $D_T$ when the stretching force is (a) between 50 and 100pN, and (b) higher than 150pN. Since the same membrane model and simulation set up as Siguenza et al. are used in our study, it is reasonable to hypothesize that the change of shear modulus may lead to similar results. The comparison for case 1 with
different shear modulus is shown in Figure 6. With larger shear modulus, the resistance to stretching increases. Thus the values of $D_A$ decrease while the values of $D_T$ increase. The discrepancy in $D_A$ between present results and those of Siguenza et al $^8$ might be due to the larger global area modulus ($1000G$) we used. As for the transverse diameter $D_T$, it shows similar trend as the study of Siguenza et al $^8$ as expected.

![Figure 6](image)

Figure 6 Comparison of simulation results with experimental results for case 1 with different shear modulus.

The comparison of simulation results with experimental results for case 1 and case 8 is shown in Figure 7. It can be seen that the results of case 8 is also in agreement with the experiments. Although there is no significant effect of the RSL on the evolution of $D_A$ and $D_T$, a slightly better agreement with the experiment is nonetheless observed when considering a biconcave RSL. However, this better agreement is achieved in the current parameter space ($G = 2.5 \times 10^{-6} \text{ N/m}$, $C = 0.5$, $k_S = 1000G$, $E_B = 6 \times 10^{-19} \text{ J}$). As shown in Figure 6 the change of parameter has a great effect on simulation results. Thus we cannot come to the conclusion that RBCs have biconcave RSL.

![Figure 7](image)

Figure 7 Comparison of simulation results with experimental results for case 1 and case 8. The shear modulus is set as $G = 2.5 \times 10^{-6} \text{ N/m}$.

The stretching test has been used by many researchers as validation of membrane model and numerical method. For example, Fedosov et al $^9$ used coarse-grained spectral-link membrane
model and dissipative particle dynamics method; Peng et al\textsuperscript{10} used a multiscale membrane model and boundary integral method; Sinha and Graham\textsuperscript{7} used continuum membrane model and boundary integral method; Zavodszky et al\textsuperscript{11} used a new material model for the membrane and discrete element method. Although these simulation results show similar trend as the experiments and even remain within the experimental error bars, none of them can match the experimental average values within the whole 0-200 pN range, especially for the transverse diameter \(D_T\). The average values of \(D_T\) are matched only for small stretching force (within 0-100 pN) or for large stretching force (within 100-200 pN). Fedosov et al\textsuperscript{9} proposed that the discrepancy between simulation and experiment may come from rotation of the cell around the axial direction. Since the experiments were observed from only a single direction, the rotation of the cell results in underestimation of the transverse diameter. However, there is no direct evidence for their assumption. Further experiments and simulations are needed on this interesting topic.

5. Preliminary study on the effect of the force gradient

The recovery motion is an intrinsic property of the cell, depending on its RSC and RSL. Thus the magnitude of the force gradient has no influence on it. We have conducted a preliminary study about the cell dependent on the magnitude of the force gradient for case 1, and the result is shown in Figure 8. It is seen that the evolution curves of \(|\Delta \varphi|\) and \(|\beta|\) for different force gradients are almost the same. In the manuscript the larger force gradient \((f_x = 2 \times 10^{-7} \text{ N m}^{-1})\) is used to ensure tank-treading motion for different cases.

![Figure 8 Time histories of the incline angle displacement \(|\Delta \varphi|\) (blue triangle) and phase angle \(|\beta|\) (red square) after the remove of different force gradients. The solid line denotes \(f_x = 2 \times 10^{-8} \text{ N m}^{-1}\) and the dashed line denotes \(f_x = 2 \times 10^{-7} \text{ N m}^{-1}\). The RSC and RSL are set as case 1. The comparison for case 8 after the remove of different force gradient is shown in Figure 9. At \(t = 0\), the cell under larger force gradient \((f_x = 4 \times 10^{-7} \text{ N m}^{-1})\) is greatly elongated. After the remove of shear forces, both cells recover to the resting shape synchronously. We do not show the results under force gradient \(f_x = 2 \times 10^{-8} \text{ N m}^{-1}\) because this force gradient leads to tumbling motion of the cell rather than tank-treading motion.](image)
Figure 9 Time sequences of cell motion after force gradients are removed. The numbers are dimensionless times. Only half of the membrane is shown for clarity. The small material point is located at the rim of the cell at the start of simulation. The red color denotes \( f_x = 2 \times 10^{-7} \text{ N m}^{-1} \) and the blue color denotes \( f_x = 4 \times 10^{-7} \text{ N m}^{-1} \). The RSC and RSL are set as case 8.

References
3. Z. Peng, University of California, San Diego, 2011.