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Red blood cells (RBCs) are the most abundant cellular element suspended in blood. Together with the usual biconcave-shaped RBCs, i.e., discocytes, unusual-shaped RBCs are also observed under physiological and experimental conditions, e.g., stomatocytes and echinocytes. Stomatocytes and echinocytes are formed from discocytes and in addition can revert back to being discocytes; this shape change is known as the stomatocyte-discocyte-echinocyte (SDE) transformation. To-date, limited research has been conducted on the numerical prediction of the full SDE transformation. Spring-particle RBC (SP-RBC) models are commonly used to numerically predict RBC mechanics and rheology. However, these models are incapable of predicting the full SDE transformation because the typically employed bending model always leads to numerical instability with severely deformed shapes. In this work, an enhanced SP-RBC model is proposed in order to extend the capability of this model type and so that the full SDE transformation can be reproduced. This is achieved through the leveraging of an advanced bending model. Transformed vesicle and RBC shapes are predicted for a range of reduced volume and reduced membrane area difference (MAD), and very good agreement is obtained in the comparison of predicted shapes with experimental observations. Through these predictions, vesicle and SDE transformation phase diagrams are developed and, importantly, in the SDE case, shape boundaries are proposed for the first time relating RBC shape categories to RBC reduced volume and reduced MAD.

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and the modeled surface was discretized using a mesh of regular triangular elements. The transformed shapes were predicted using the Monte Carlo method to ensure that stable equilibrium shapes were obtained. Through simulations, it was concluded that the SDE transformation can be induced by varying the MAD and, importantly, it was proposed that the equilibrium cytoskeleton shape is likely to be an ellipsoid rather than the widely adopted biconcave shape. A similar simulation study was completed by Khairy and Howard [14] where, in addition to the SDE transformation, vesicle transformation was also simulated. One of the aims of this study was to demonstrate the computational efficiency of the spherical harmonics parameterization method which was employed to predict the transformed shapes. In the study, both the vesicle and RBC membrane were modeled using continuum mechanics, i.e., the SK constitutive law [13]. Based on the results obtained, the authors claimed that their method was highly computationally efficient compared with other modeling approaches.

In both the simulation studies by Lim et al. [12] and Khairy and Howard [14], the RBC membrane was modeled using a continuum constitutive law, i.e., a continuum model, assuming the membrane was a homogeneous isotropic thin shell. In addition, an area-difference-elasticity (ADE) contribution was introduced into their membrane models to include the effect of the MAD change on the SDE transformation. As an alternative to using continuum models, some researchers have employed spring-network-based models to numerically simulate the RBC membrane [15–18]. These networks consist of a number of nonlinear springs arranged in a triangle-based topology. These RBC models are known as spring-particle (SP) RBC models and are widely used to study RBC mechanics [15], rheology [16], and RBC–related diseases [19]. SP–RBC models are mathematically simpler than continuum models and have extensive modeling capabilities. Diseased RBCs consist of a number of nonlinear springs arranged in a triangle-based topology. These RBC models are known as spring-particle (SP) RBC models and are widely used to study RBC mechanics [15], rheology [16], and RBC–related diseases [19]. SP–RBC models are mathematically simpler than continuum models and have extensive modeling capabilities.

Fig. 1 Schematic diagrams of (a) a healthy RBC and (b) the RBC membrane. The PM consists mainly of a lipid bilayer, while the cytoskeleton is a hyper-elastic-behaving network attached to the PM via anchoring proteins.

Spring-Particle Red Blood Cell Model

The SP–RBC model employs an area and volume conserving spring network to predict a RBC equilibrium structural geometry. As the model has been developed to predict steady-state geometries only, the viscous behavior of the PM does not need to be accounted for.

Geometry of the RBC Model. A healthy RBC is biconcave in shape. The average RBC surface geometry can be approximated by the equation [26]

\[
z = 2D_0 \left(1 - \frac{4(y^2 + z^2)}{D_0^2}\right)^{1/2} \left(a_0 + a_1 \frac{x^2 + y^2}{D_0^2} + a_2 \frac{x^2 + y^2}{D_0^2} \right)
\]

(1)

where \(x, y,\) and \(z\) are the Cartesian coordinates; \(D_0\) is the initial diameter of the RBC with a value of 7.82 \(\mu\)m; and \(a_0, a_1,\) and \(a_2\) are constants with values of 0.0518, 2.026, and –4.491, respectively. This equation is employed in SolidWorks (SolidWorks Corp., Concord, MA) to generate a 3D solid model. The surface of this solid model is subsequently discretized using Ansys (Ansys Inc., Canonsburg, PA) to obtain a mesh of triangular elements. This mesh network forms the spring-particle system, where the element boundaries are considered the springs and the element nodes considered the particles. This spring-particle system represents the neutral plane of the RBC membrane, located at the interface of the inner and outer leaflet surfaces of the PM bilayer, see Fig. 1.

Helmholtz Free Energy of the RBC Model. Structurally, a RBC is a liquid-core membrane-bounded capsule. The liquid, known as the cytoplasm, is an incompressible Newtonian fluid with constant volume. The membrane has a dual-layer structure consisting of the PM and the cytoskeleton. Mechanically, the PM confers resistance to surface area dilation and out-of-plane

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bending, while the cytoskeleton confers resistance to in-plane 
4 bending. Correspondingly, the Helmholtz free energy of the RBC 
6 model has four constituent elements: energies due to constraints 
8 of the model volume and membrane surface area, out-of-plane 
10 bending, and in-plane shearing, i.e.,

\[ E_{\text{total}} = E_{\text{V}} + E_{\text{AC}} + E_{B} + E_{S} \]  

where \( E_{\text{total}} \), \( E_{\text{V}} \), \( E_{\text{AC}} \), \( E_{B} \), and \( E_{S} \) are the energy terms for the overall RBC model, cytoplasm volume constraint, PM surface-

area constraint, PM out-of-plane bending, and cytoskeleton in-

plane shearing, respectively. It should be noted that each of these 
0 energy terms is relative to a reference configuration.

**Constraint Energies.** The cytoplasm volume-constraint energy 
2 of the RBC model is given by

\[ E_{\text{V}} = \frac{K_{v}}{2 \lambda_{o}} (V - V_{o})^{2} \]  

where \( K_{v} \) is the volume-constraint modulus \([27,28]\) and in this 
4 work is set as 1000 N/m², \( V \) and \( V_{o} \) are the RBC-model instantaneous 
6 and reference volumes, respectively.

The PM surface-area constraint energy consists of two terms. 
0 The first one is due to the movement restriction imposed by the 
1 anchoring proteins that attach the cytoskeleton to the PM, while 
3 the second one is due to the surface-area incompressibility of the 
5 PM, i.e.,

\[ E_{\text{AC}} = \frac{K_{LA}}{2} \sum_{i=1}^{N_{i}} \frac{(A_{i} - A_{i,o})^{2}}{A_{i,o}} + \frac{K_{GA}}{2A_{o}} (A - A_{o})^{2} \]  

where \( K_{LA} \) and \( K_{GA} \) are the local and global area dilation moduli 
2 \([27,28]\) which are set as \( 10^{-4} \) N/m and \( 10^{-7} \) N/m, respectively, \( k \) 
5 is triangular element index, \( N_{i} \) is the total number of triangles, \( A_{i,o} \) 
7 and \( A_{o} \) are instantaneous and the reference areas of triangle \( i \), 
9 respectively, and \( A \) and \( A_{o} \) are instantaneous and reference surface 
11 areas of the RBC model, respectively.

**Bending Energy.** Typically, the bending model employed by 
2 conventional SP-RBC models is an approximation to the Helfrich 
3 bending model \([25]\) resulting from a number of assumptions, see 
5 the Appendix. Importantly, the bending model is based on the 
7 sub-tended angle between neighboring triangular elements and is 
9 mesh dependent \([29]\). Consequently, the conventional SP-RBC 
11 model is incapable of simulating the SDE transformation, because 
13 the bending model causes numerical instability as the model 
15 membrane undergoes severe deformation.

The membrane bending model employed in this work is based 
0 on the one previously employed by Lim et al. \([12]\). This bending 
2 model energy consists of two terms. The first term is due to the 
4 local bending resistance, while the second term is due to the membrane 
6 ADE contribution \([25]\). In contrast to the conventional one, 
8 the employed bending model is based on surface curvature, i.e.,

\[ E_{B} = \frac{K_{LB}}{2} \sum_{j=1}^{N_{j}} \left[ A_{j}(C_{j} - C_{j,o})^{2} \right] - \frac{K_{GB}}{P_{A_{o}} \lambda_{o}} [\Delta A - \Delta A_{o}]^{2} \]  

where \( K_{LB} \) and \( K_{GB} \) are the RBC-membrane local and global 
2 bending moduli \([25]\), respectively, with the same default value of 
4 \( 2.5 \times 10^{-19} \) N m, \( i \) is particle index, \( N_{p} \) is the total number of 
6 particles, \( A_{i} \) is area occupied by particle \( i \), \( C_{i} \) and \( C_{i,o} \) are instantaneous 
8 and reference curvatures of particle \( i \), respectively, \( H \) is the 
0 membrane thickness with a value of \( 4 \times 10^{-7} \) m, and \( A_{i} \) and \( \Delta A_{o} \) 
1 are the instantaneous and reference MADs, respectively. The par-

**Shearing Energy.** The membrane shearing energy is the sum of 
0 the potential energies of the springs comprising the network 
2. The springs employed are worm-like-chains (WLC) and, 
4 therefore, the shearing energy has the following form:

\[ E_{S} = k_{g}g \frac{1}{4p} \sum_{j=1}^{N_{j}} \left[ L_{j} \left( \frac{3r_{j}^{2} - 2r_{j}^{3}}{1 - r_{j}^{2}} - 4c_{j}r_{j}^{2} - c_{2} \right) \right] \]  

where \( k_{g} \) is the Boltzmann constant, \( T \) is the absolute temperature, 
0 \( p \) is the persistent length of the WLC elements, \( j \) is spring index, 
2 \( N_{j} \) is the total number of springs, \( L_{j} \) is the contour length of 
4 WLC element \( j \) and is defined as \( 2.3 \) times the reference element length \( L_{j_{ref}} \) and \( r_{j} \) is the ratio of the instantaneous element length 
6 \( L_{j} \) to the contour length of spring \( j \), i.e., \( r_{j} = L_{j}/L_{j_{ref}} \). The WLC- 
8 energy equation mentioned earlier differs from the conventional 
0 form as two constants \( c_{1} \) and \( c_{2} \) are introduced so that the spring 
1 elements have initial lengths. The constants \( c_{1} \) and \( c_{2} \) are set as 
3 0.9673 and \(-0.9698\), respectively, from the definition of contour 
5 length \([30]\).

**Initial and Reference Model Configurations.** The RBC 
2 model is used to predict the transformed shape of both vesicles 
4.

**Fig. 2** Illustration of the curvature of particle \( i \). The shaded 
6 area is the area occupied by particle \( i \), i.e., \( A_{i} \). \( L_{j} \) is the length of 
8 edge \( j \), \( n_{i} \) and \( n_{o} \) are the normal vectors to the two triangular 
0 elements which share edge \( j \), and \( \theta_{j} \) is the angle formed by 
2 these vectors, i.e., the included angle.
Potential must equal the change of the model free energy, i.e.,

\[ U = E_{\text{Total}, \sigma + 1} - E_{\text{Total}, \sigma} \]

where \( E_{\text{Total}, \sigma + 1} \) and \( E_{\text{Total}, \sigma} \) are the model free energies after and before the incremental displacement of all the particles, respectively. This equation implies that the model free energy reduces on successive incremental displacements of the particles until the particle forces reduce to zero. In this work, a transformed RBC shape with a minimum free energy is probed by iteratively obtaining the internal force on each particle and incrementing each particle position by \( d \mathbf{s} \), until for all nodes \( |F| < 10^{-22} \) N. The incremental displacement \( d \mathbf{s} \) is calculated as follows for each node:

\[ d \mathbf{s} = \alpha \mathbf{F} \]

where \( \alpha \) (10^5 m/N) is a model constant with a magnitude equal to 10% of the smallest spring-element length in the model.

Results and Discussion

This section contains results from two test cases. The first test case involves the prediction of transformed vesicle shapes, while the second one involves the prediction of transformed SDE RBC shapes. In both test cases the transformed shapes are obtained for a range of model volume and MAD.

Prediction of Vesicle Transformation.

Physically, a vesicle is equivalent to a RBC without the cytoskeleton. Therefore, in the model, the free energy term related to the cytoskeleton, i.e., shear-driven energy, is not required, with the vesicle transformation being driven primarily by minimization of the bending energy. This is an excellent test case to examine the accuracy and stability of the bending model. In this subsection, the model is first validated by comparing predicted vesicle shapes with experimental observations. Then, a vesicle transformation phase diagram is presented showing transformed vesicle shapes for a range of model volume and MAD, so that the capability of the model can be examined.

Vesicle Model Validation.

Several predicted transformed vesicle shapes, for various model volumes and MADs, are compared with experimental observations [33] in Table 1. To make the results transferable to vesicles with different surface areas, both the volume and MAD are nondimensionalized with respect to a sphere with the same surface area and membrane thickness as the initial configuration. The nondimensionalized volume, known as the reduced volume, is given by

\[ v = \frac{V_{\text{vesicle}}}{4/3\pi R_0^3} \]

where \( V_{\text{vesicle}} \) is the vesicle volume and \( R_0 \) is the sphere radius equal to 3.265 \( \mu \)m for a surface area of 134 \( \mu \)m². The nondimensional MAD is given by

\[ \nu = 

Fig. 3 Comparison of the geometry of (a) the initial discocyte with (b) the ellipsoid which is used to define the reference spring lengths, \( L_{\sigma, j} \), for the WLC springs. The magnified sections show the equivalent spring in both meshes.

and RBCs for a wide range of model volume and MAD. The initial vesicle/RBC configuration is always taken as a discocyte with a volume, area, and MAD of approximately 94 \( \mu \)m³, 134 \( \mu \)m², and 0.332 \( \mu \)m², respectively. The reference configuration is defined as follows: \( V \) is the reference volume with a value specified as the desired/target volume by the user, \( A \), is the reference surface area and in this work is specified as the desired/target area of the discocyte, \( \Delta A \), is the reference area with a value specified as the desired/target MAD by the user, \( C_j \), is the reference nodal curvature for node \( j \) with all nodes assigned the same value derived from \( \Delta A \), i.e., Eq. (7), and \( L_{\sigma, j} \) is the reference length for spring \( j \) on an ellipsoid [25] with a volume of 95% of a sphere with the same surface area as the initial configuration, see Fig. 3. Experimental measurements suggest that the relaxed cytoskeleton geometry is a quasi-sphere [31], while numerical predictions by Lim et al. [25] suggest that the most likely relaxed geometry is an ellipsoid [25], as described earlier and is thus used in this work.

RBC Shape Prediction. A transformed RBC shape is predicted using the principle of minimum free energy. In the RBC model, the free energy depends solely on the geometrical distribution of the particles since viscosity is neglected, i.e.,

\[ E_{\text{Total}} = f(s_1 + s_2 + \ldots + s_i) \]

or

\[ F_{\sigma, i} = -\frac{\partial E_{\text{Total}}}{\partial x_i}, F_{\sigma, j} = -\frac{\partial E_{\text{Total}}}{\partial y_j}, \text{ and } F_{\sigma, k} = -\frac{\partial E_{\text{Total}}}{\partial z_k} \]

where \( F_{\sigma, i} \), \( F_{\sigma, j} \), and \( F_{\sigma, k} \) are the components of the internal force \( F \), exerted on particle \( i \) in the \( x \), \( y \), and \( z \) directions, respectively. The work done by the internal force on particle \( i \), known as the force potential [32], for an infinitesimal displacement in the direction of the force is simply

\[ \Phi_i = F_i ds_i \]
where $H$ is the membrane thickness with a value of 4 nm. Note that the initial vesicle configuration, therefore, has $v = 0.645$ and $\Delta a = 1.01$ [34,35]. These predicted vesicle shapes were obtained using two different meshes: a fine mesh with 4636 triangular elements and a coarse mesh with 1604 elements. By observation, it can be seen that the predicted transformed shapes using either mesh are very similar to the observed vesicle shapes. Some differences are observable with the coarse-mesh models, however, and are due to the fact that the mesh elements are too large to correctly define the curved surface. The close agreement with the observed shapes indicates that the membrane bending energy is being correctly calculated and, importantly, the calculation is independent of mesh density.

**Phase Diagram of Vesicle Transformation.** The fine-mesh model described earlier was used to predict transformed vesicle shapes for a range of reduced volume and MAD, see Fig. 4. It can be seen from this figure that the predicted shapes fall within the shape boundaries proposed by Seifert et al. [36]. These boundaries separate the vesicle shapes into several categories, e.g., pear, dumbbells, and stomatocytes. Seifert et al. investigated vesicle shape transformation using a numerical model. In their study, the phase diagram of vesicle transformation was developed using a bilayer-coupling model, minimization of the bending energy only, and with constraints similar to those employed in this work. Interestingly, however, the membrane reference curvature was always

### Table 1: Comparison of experimentally observed and numerically predicted transformed vesicle shapes using a fine and a coarse mesh. The experimental images are reprinted with permission from Refs. [34] and [35].

<table>
<thead>
<tr>
<th>Reduced Vol.</th>
<th>MAD</th>
<th>Experimentally observed</th>
<th>Numerically predicted (4636 elements)</th>
<th>Numerically predicted (1604 elements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.60</td>
<td>0.53</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>0.60</td>
<td>0.65</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>0.60</td>
<td>0.95</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
<tr>
<td>0.86</td>
<td>1.1</td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td>0.85</td>
<td>1.2</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
</tr>
<tr>
<td>0.84</td>
<td>1.2</td>
<td><img src="image16" alt="Image" /></td>
<td><img src="image17" alt="Image" /></td>
<td><img src="image18" alt="Image" /></td>
</tr>
</tbody>
</table>
Prediction of SDE Transformation. In this subsection, the RBC model is first validated by comparing predicted transformed RBC shapes both with experimental observations and with predicted results using a continuum RBC model. Then, an SDE transformation phase diagram is presented showing predicted transformed shapes for a range of reduced volume and reduced MAD.

RBC Model Validation. Numerically predicted SDE transformed shapes are compared both with experimentally observed shapes and those predicted using a continuum RBC model [12,14] in Table 2. This comparison is for a reduced volume $v \approx 0.645$ and for a range of reduced MAD. By observation, it can be seen that a very good qualitative agreement is achieved. Overall, the predicted shapes accurately capture the detailed transformed geometries. In addition, it can be seen that, with shearing energy included, the model is capable of predicting the severely deformed RBCs that occur for high reduced MADs. The good agreement indicates that the SP-model can predict RBC mechanics upon severe deformation and, importantly, the predicted results are equivalent to those predicted using a continuum model [12,37–39].

These transformed shapes were predicted using a mesh with 8610 triangular elements. This mesh density was chosen following a mesh convergence study that had the aim of establishing the mesh that would produce smooth and comparable shapes to those observed and those predicted using the continuum model. Figure 5 shows some results from this study showing predicted echinocytes II with a reduced volume $v \approx 0.645$ and a reduced MAD $\Delta \tau \approx 1.66$. These echinocytes were predicted using meshes with 2664, 4636, 8610, and 12,930 triangular elements. When using meshes with less than 8610 elements some of the spicules are very sharp tipped, see Figs. (5(a) and (5(b)). However, when using meshes with 8610 or more elements, all the spicules are very smooth, and very little difference is found between the predicted shapes. Also, it is interesting that these four echinocytes have a comparable number of spicules; the one predicted with 4636 elements has 25 spicules while the others have 26 spicules.

Phase Diagram of SDE Transformation. The SP-RBC model with a mesh of 8160 triangular elements was used to obtain the phase diagram of SDE transformation. Figure 6 shows predicted transformed RBC shapes with reduced MADs of one and above, and at reduced volumes of 0.55, 0.645, 0.75, and 0.85. These predicted shapes correspond to the transformation of discocytes to echinocytes I, II, and III. From observation, it can be seen that the size of the echinocytes I spicules are slightly different for RBCs with different reduced volumes. Low reduced-volume RBCs tend to have a higher number of periodic undulations on the rim, e.g., 11 undulations for the RBC with a reduced volume of 0.55, but eight for the one with a reduced volume of 0.85. It can also be seen that the size of those undulations are approximately the same as the thickness of the RBC rim. Therefore, high reduced-volume RBCs, which have thick rims, have fewer undulations compared with those with low reduced volumes. With further increases of the MAD from echinocyte I, the undulations are differentiated into small spicules distributed evenly over the entire RBC surface to form echinocytes II. With an even further increase of the MAD, echinocytes II transform to echinocytes III as more spicules are formed and the oblate-shaped body of the echinocytes II is transformed into an ellipsoidal shape. This body shape of the echinocytes III is affected by the choice of the reference spring length, since the membrane shearing energy is the dominant energy determining the RBC shape transformation. In addition, it can be seen in Fig. 6 that echinocytes II form in an extremely small range of MAD in RBCs of high reduced volume. In those RBC models, the echinocyte body is transformed into an ellipsoidal shape before echinocytes II form and the spicules remain at the echinocyte rim.

In a similar fashion, Fig. 7 presents predicted transformed RBC shapes with reduced MADs of one and below, and at reduced volumes of 0.55, 0.645, 0.75, and 0.85. These predicted shapes correspond to the shape transformations of discocytes to stomocytes I, II, and III. Stomatocytes I form when one of the concave invaginations vanishes, while the other becomes deeper. With further decrease of the MAD, inward-directed undulations form in the remaining concave MAD around $\Delta \tau \approx 1.0$. Second, the shape transformation is more severe for the model with high reduced volume than the one with low reduced volume. Third, the shape categories for both transformations are similar, e.g., stomatocytes...
Table 2  Comparison of experimentally observed and numerically predicted transformed RBC shapes in the SDE transformation. The predicted shapes are also compared with those predicted using a continuum model [12]. The experimental images are reprinted with permission from Refs. [37–39].

<table>
<thead>
<tr>
<th>Shape Category</th>
<th>Reduced Vol.</th>
<th>Reduced MAD</th>
<th>Experimentally Observed</th>
<th>Numerically Predicted (SP–RBC)</th>
<th>Numerically Predicted (Continuum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatocyte III</td>
<td>0.645</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatocyte II</td>
<td>0.645</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatocyte I</td>
<td>0.645</td>
<td>0.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discocyte</td>
<td>0.645</td>
<td>1.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echinocyte I</td>
<td>0.645</td>
<td>1.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echinocyte II</td>
<td>0.645</td>
<td>1.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echinocyte III</td>
<td>0.645</td>
<td>1.66</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5  Predicted echinocytes III with a reduced volume \( v = 0.645 \) and a reduced MAD \( \Delta a = 1.66 \). These transformed shapes were predicted using meshes with (a) 2664, (b) 4636, (c) 8610, and (d) 12,930 triangular elements.
vesicle and stomatocyte RBC, dumbbells and echinocyte II, and pears and echinocyte III. From observation, RBC shape boundaries can be defined in the SDE-transformation phase diagrams. These boundaries separate the predicted RBC geometries into shape categories, i.e., stomatocytes, discocytes, and echinoctyes, see Figs. 6 and 7. However, it should be noted that the predicted RBC shapes may be metastable shapes due to the complexity of the RBC model [25] and that these boundaries are based on the assumptions and conditions employed in this work.

Conclusion

In this work, an enhanced SP-RBC model is proposed in order to extend the capability of conventional spring-network-based RBC models. In contrast with conventional SP-RBC models, in the enhanced model, the membrane bending energy is calculated using the surface curvature, thus rendering it independent of the mesh topology and the degree of model deformation. In addition, an ADE contribution to the bending energy is added for the first time in a SP-RBC model.

The SP-RBC model is used to predict transformed RBC shapes. While the results presented here can be considered as a follow-on to the work of Lim et al. [25], there are several differences: in this work, the RBC model employed is spring-network-based while Lim’s was continuum based, the accuracy of the membrane bending model is first established via a vesicle transformation study, and the effect of model volume on the transformed shapes is assessed, whereas in Lim et al., only one model RBC volume was used. Critically, in this work, SDE RBC shape boundaries are proposed for the first time which relate RBC shape categories to RBC reduced volume and reduced MAD.

The results presented here show that the enhanced SP-RBC model can accurately simulate vesicle and RBC SDE shape transformations. Importantly, very good agreement is observed in comparisons of numerically predicted shapes with experimental observations. In addition, the predicted SDE shapes are also very similar to those predicted using a continuum RBC model indicating the equivalence of the present SP-RBC model with continuum models.

Acknowledgment

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Nomenclature

\( a_0, a_1, a_2 = \) red blood cell geometry constants, \( 10^{-6} \) m
\( A, \Delta A = \) area, area difference, \( \text{m}^2 \)
\( c_1, c_2 = \) worm-like-chain constants, (N, J)
\( C = \) curvature, \( \text{m}^{-1} \)
\( D = \) diameter, m
\( E = \) energy, J
\( F, F = \) force, force vector, N
\( H = \) thickness, m
\( k_b = \) Boltzmann constant, \( 1.381 \times 10^{-23} \text{ m}^2 \text{kg}/(\text{s}^2 \text{K}) \)
\( k_g = \) bending rigidity, J
\( K_{LA}, K_{LGA} = \) local, global area dilation moduli, J/m
\( K_{LR, GB} = \) local, global bending modulus, J
\( K_v = \) volume constraint modulus, J/m^2
\( L, L = \) length, contour length, m
\( N_p, N_x, N_T = \) number of particles, springs, triangles, springs
\( p = \) persistent length of worm-like-chain, m
\( r = \) stretch ratio, m/m
\( R = \) radius, m
\( s = \) cartesian coordinate vector, m
\( T = \) absolute temperature, K
\( V = \) volume, m^3
\( \Delta A = \) reduced/dimensionless area difference, \( \text{m}^2/\text{m}^2 \)

Greek Symbols

\( \Phi = \) force potential, J
\( \theta = \) angle, radian
\( \mu = \) micron, \( 10^{-6} \)
\( \xi, \zeta = \) normal vectors, m

Subscripts or Superscripts

\( AC = \) area dilation constraint
\( B = \) bending constraint
\( i = \) particle index
\( j = \) spring index
\( k = \) triangle index
\( o = \) spontaneous/initial value
\( S = \) shearing constraint
Total = total
V, VC = volume, volume constraint

Aberrations widely used in text
ADE = area difference elasticity
MAD = membrane area difference
PM = plasma membrane
RBC = red blood cell
SDE = stomatocyte-discocyte-echinocyte
SK = skak
SP = spring particle
WLC = worm-like-chain

Appendix
According to the Helfrich bending model, the bending energy, $E_B$, contained in a thin membrane element of area $A$ at its neutral plane, see Fig. 8, is given by

$$ E_B = \frac{K_B}{2} (C - C_o)^2 A \quad (A1) $$

where $K_B$ is the bending modulus, $C$ is the curvature, $C_o$ is the curvature of a reference configuration, with both curvatures assumed constant over the surface. For the element shown in Fig. 8, the curvature is calculated as $C = 1/R_1 + 1/R_2$, where $R_1$ and $R_2$ are the principal radii of curvature at the neutral plane.

For the membrane element, it can also be shown that the curvature can be calculated from the area difference between inner and outer surfaces of the membrane, $\Delta A$, i.e.,

$$ C = \frac{\Delta A}{A} \quad (A2) $$

where $H$ is the element thickness, assumed constant.

In our spring-particle model the membrane surface is discretized using a mesh of contiguous triangular elements, with the element boundaries considered the springs and the element nodes considered the particles. The model bending energy is calculated at the nodes/particles, and for each particle is the energy of a region of the membrane centered around that particle. Consider the four triangular elements (of the overall surface mesh) shown in Fig. 9 used to model the membrane element shown in Fig. 8, with a combined area of $A$ and sharing the common particle $i$. These elements are assumed to be located at the neutral plane of the membrane. Neighboring elements have a shared edge of length $L_j$, where $j$ is the index of the edge. In addition, the surface-normal vectors to neighboring elements form the included angle $\theta_j$.

Using Eq. (A1), the model bending energy for particle $i$ is calculated as follows:

$$ E_{B,i} = \frac{K_B}{2} (C_i - C_{i,o})^2 A_i \quad (A3) $$

where $C_i$ and $C_{i,o}$ are the instantaneous and reference curvatures at particle $i$, and $A_i$ is the effective area of particle $i$ equal to one-third of the total area of the elements which share the particle.

Using Eq. (A2), the curvature $C_i$ is calculated as

$$ C_i = \frac{1}{2} \sum_{j=1}^{N_w} (\theta_j L_j) \quad (A4) $$

where $N_w$ is the total number of element edges meeting at particle $i$. Equation (19) combined with Eq. (A4) are the equations employed in this work to calculate the bending energy. Summing the bending energies of all the particles gives the bending energy of the entire membrane model.

For the case in Fig. 8, where one of the principal radii of curvature, e.g., $R_3$, is infinitely large, the membrane element can be modeled by two planar elements as shown in Fig. 10, with a shared edge of length $L$ and a combined area $A$.

Using Eq. (A1), the model bending energy for these two elements is

$$ E_B = \frac{K_B}{2} (C - C_o)^2 A \quad (A5) $$

with the model curvature given by

$$ C = \frac{\theta L}{A} \quad (A6) $$
Therefore, the model bending energy can be calculated by substituting Eq. (A6) into Eq. (A5), i.e.,
\[ E_B = \frac{k_B}{2} \left( \frac{(\theta - \theta_0)^2 L^2}{A} \right) \]
\[ \text{(A7)} \]
where \( \theta_0 \) is the corresponding angle of the reference configuration.

The bending energy of the entire model is obtained by calculating the bending energy of the element pairs.

Using Taylor’s Theorem which states the following:
\[ \cos(\theta - \theta_0) = 1 - \frac{1}{2!}(\theta - \theta_0)^2 + \frac{1}{4!}(\theta - \theta_0)^4 - \frac{1}{6!}(\theta - \theta_0)^6 + \cdots \]
\[ \text{(A8)} \]
the \((\theta - \theta_0)^2\) term in Eq. (A8) can be replaced so that the bending energy can be written as
\[ E_B = \frac{k_B}{A} \left[ 1 - \cos(\theta - \theta_0) + \frac{1}{4!}(\theta - \theta_0)^4 \right. \]
\[ \left. - \frac{1}{6!}(\theta - \theta_0)^6 + \cdots \right] \]
\[ \left( \frac{-1}{2!} \right)^n (\theta - \theta_0)^2n \]
\[ \text{(A9)} \]
If both of the element models are equilateral triangles and if all the element edge lengths remain constant during deformation, the bending energy can be further simplified to
\[ E_B = \frac{k_B}{3} \left[ 1 - \cos(\theta - \theta_0) + \frac{1}{4!}(\theta - \theta_0)^4 \right. \]
\[ \left. - \frac{1}{6!}(\theta - \theta_0)^6 + \cdots \right] \]
\[ \text{(A10)} \]
\[ \text{or} \]
\[ E_B = k_B \left[ 1 - \cos(\theta - \theta_0) + \frac{1}{4!}(\theta - \theta_0)^4 \right. \]
\[ \left. - \frac{1}{6!}(\theta - \theta_0)^6 + \cdots \right] \]
\[ \text{(A11)} \]
where \( k_B = 2k_B/\sqrt{3} \).

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\[ \text{Vol. 00, MONTH 2017} \]

\[ \text{Page: 10} \]

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