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An Enhanced Spring-Particle Model for Red Blood Cell Structural Mechanics: Application to the Stomatocyte–Discocyte–Echinocyte Transformation

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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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15 Introduction

Red blood cells (RBCs) are the most abundant cellular element suspended in blood. A normal RBC count in a healthy individual is about 5,000,000/mm³, accounting for 99% of all suspended cellular elements or 45% of the total blood volume. These RBCs are highly deformable and, during their lifespan of about 120 days, repeatedly travel through blood vessels with diameters as small as 3 μ m delivering oxygen in the circulatory system [1]. A healthy RBC has a biconcave shape with a diameter of 8 μ m, a thickness of 2 μ m, and a volume of 65% of a sphere with the same surface area. Structurally, a RBC is a liquid-core membrane-bounded capsule. The liquid, known as the cytoplasm, is a mixture of hemoglobin and enzymes and is generally considered as a Newtonian fluid [2]. The membrane is a composite material consisting of an outer layer of plasma membrane (PM) and an inner cytoskeleton layer. The PM consists mainly of a lipid bilayer which offers no resistance to in-plane shearing, a small resistance to out-of-plane bending but a high resistance to surface area dilation. The cytoskeleton is a nonlinear hyper-elastic-behaving network, attached to the PM via anchoring proteins, conferring structural integrity to the RBC membrane (see Fig. 1).

Besides the normal biconcave-shaped RBCs, known as discocytes, some unusual-shaped RBCs are also observed under physiological [3–5] and experimental conditions [6–8], e.g.,

stomatocytes and echinocytes. Instead of having a biconcave shape, stomatocytes have a single invagination, while echinocytes have spicules evenly distributed on the membrane surface. Stomatocytes and echinocytes are formed from discocytes and can revert back to being discocytes; this shape change is known as stomatocyte–discocyte–echinocyte (SDE) transformation. The SDE transformation can be induced by a number of factors, e.g., deviations from healthy ranges of pH and cholesterol levels. A deviation from the healthy range of pH level promotes ionic interactions on the PM surface [9,10], while a deviation in the cholesterol level promotes lipid and protein exchanges between the two leaflets of the PM bilayer [11]. SDE transformation inducing factors lead to a change in the volumes of the bilayer leaflets, and since the leaflet thicknesses are constant, these volume changes result in a change of the area difference between the outside surface of the outer layer and inside surface of inner layer, i.e., a change of the membrane area difference (MAD). In other words, every particular RBC shape in the SDE transformation is associated with a unique combination of MAD, volume and area, and with the large variety of SDE transformation shapes possible, the use of the MAD, in addition to the volume and area, allows unique RBC shapes to be identified.

To-date, limited research has been conducted on the numerical simulation of the full SDE transformation. Lim et al. [12] simulated the full transformation of a normal RBC. This simulation study assessed the effects of both the MAD and the equilibrium cytoskeleton shape on the SDE transformation for one volume only, i.e., the normal RBC volume. In this study, the RBC membrane was modeled using the Skalak (SK) constitutive law [13]

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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.

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 computational 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 RBC dynamics [20] and RBC fluctuations [21,22] have been successfully simulated using SP-RBC models, while no similar simulations have been conducted using continuum models. However, the Spring-particle RBC (SP-RBC) models developed to-date are incapable of simulating the SDE transformation while the continuum-based models are. This deficiency is due to the bending model typically employed, which is based on the subtended angle between neighboring network triangular elements. Use of this bending model to predict the full SDE transformation leads to numerical instability always when modeling echinocytes and sometimes with stomatocytes. The use of very fine meshes does not alleviate the instability, even when using a very fine mesh with 60,000 spring elements. Mesh reconstruction may be a solution but would render the model very demanding computationally.

Inspired by previous research [12,14], an enhanced SP-RBC model is proposed here in order to improve the capability of the conventional ones. This SP-RBC model is more advanced than the conventional ones [23,24] from two perspectives. First, the bending model, which uses surface curvature, is independent of the network topology. Second, an ADE contribution, based on the MAD change, is included. This enhanced SP-RBC model is used to predict the transformed shapes of vesicles and RBCs resulting from changes to the model volume and MAD. Predictions of

transformed vesicle shapes facilitate an examination of the accuracy and stability of the bending model, since vesicle transformation is driven mainly by minimization of the bending energy. Importantly, the RBC predictions can be considered as a follow-on to the work of Lim et al. [25], with an extensive investigation of the effect of changing the RBC volume completed. In addition, a direct comparison of vesicle and RBC transformed shapes yields RBC shape boundaries, which relate RBC shape categories to RBC reduced volume and reduced MAD. In Sec. 2, the structural mechanics employed by the SP-RBC model are introduced along with the methodology employed for predicting a RBC equilibrium shape. In Sec. 3, results of two test cases are presented. The first test case involves the prediction of transformed vesicle shapes, while the second test case involves the prediction of the full SDE transformation resulting from changes to the RBC volume and MAD.

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]

$$z = \pm D_o \left(1 - \frac{4(x^2 + y^2)}{D_o^2} \right)^{1/2} \left(a_0 + a_1 \frac{x^2 + y^2}{D_o^2} + a_2 \frac{(x^2 + y^2)^2}{D_o^4} \right) \quad (1)$$

where x , y , and z are the Cartesian coordinates; D_o is the initial diameter of the RBC with a value of $7.82 \mu\text{m}$; 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

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

$$E_{\text{Total}} = E_{\text{VC}} + E_{\text{AC}} + E_B + E_S \quad (2)$$

where E_{Total} , E_{VC} , E_{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 energy terms is relative to a reference configuration.

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

$$E_{\text{VC}} = \frac{K_V}{2V_o} (V - V_o)^2 \quad (3)$$

where K_V is the volume-constraint modulus [27,28] and in this work is set as 1000 N/m^2 . V and V_o are the RBC-model instantaneous and reference volumes, respectively.

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

$$E_{\text{AC}} = \frac{K_{\text{LA}}}{2} \sum_{k=1}^{N_T} \frac{(A_k - A_{k,o})^2}{A_{k,o}} + \frac{K_{\text{GA}}}{2A_o} (A - A_o)^2 \quad (4)$$

where K_{LA} and K_{GA} are the local and global area dilation moduli [27,28] which are set as 10^{-4} N/m and 10^{-3} N/m , respectively, k is triangular element index, N_T is the total number of triangles, A_k and $A_{k,o}$ are instantaneous and the reference areas of triangle k , respectively, and A and A_o are instantaneous and reference surface areas of the RBC model, respectively.

Bending Energy. Typically, the bending model employed by conventional SP-RBC models is an approximation to the Helfrich bending model [25] resulting from a number of assumptions, see the Appendix. Importantly, the bending model is based on the subtended angle between neighboring triangular elements and is mesh dependent [29]. Consequently, the conventional SP-RBC model is incapable of simulating the SDE transformation, because the bending model causes numerical instability as the model membrane undergoes severe deformation.

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

$$E_B = \frac{K_{\text{LB}}}{2} \sum_{i=1}^{N_p} [A_i (C_i - C_{i,o})^2] + \frac{K_{\text{GB}}}{H^2 A_o} [\Delta A - \Delta A_o]^2 \quad (5)$$

where K_{LB} and K_{GB} are the RBC-membrane local and global bending moduli [25], respectively, with the same default value of $2.5 \times 10^{-19} \text{ N-m}$, i is particle index, N_p is the total number of particles, A_i is area occupied by particle i , C_i and $C_{i,o}$ are instantaneous and reference curvatures of particle i , respectively, H is the membrane thickness with a value of $4 \times 10^{-9} \text{ m}$, and ΔA and ΔA_o are the instantaneous and reference MADs, respectively. The particle area, A_i , is one-third of the total area of the triangles that share particle i and the curvature, C_i , is calculated as

$$C_i = \frac{\frac{1}{2} \sum_{j=1}^{N_w} (\theta_j L_j)}{A_i} \quad (6)$$

where j is the element edge index, N_w is the total number of edges which share particle i , L_j is the length of edge j , and θ_j is the angle between the triangles which share edge j , see Fig. 2.

The MAD, ΔA , is related to the curvature as follows:

$$\Delta A = H \sum_{i=1}^{N_p} (C_i A_i) \quad (7)$$

By substituting Eq. (7) into Eq. (5), it is interesting to see that the membrane thickness cancels out so that the thickness can be neglected. Upon cancellation, it is noted the bending energy due to the ADE contribution has a similar form to that of the local bending resistance and these energies could be lumped together [12]. However, the ADE contribution is explicitly retained in the bending energy model in this work as a measurement to identify the model shape.

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

$$E_S = \frac{k_B T}{4p} \sum_{j=1}^{N_s} \left[L_{c,j} \left(\frac{3r_j^2 - 2r_j^3}{1 - r_j} - 4c_1 r_j - c_2 \right) \right] \quad (8)$$

where k_B is the Boltzmann constant, T is the absolute temperature, p is the persistent length of the WLC elements, j is spring index, N_s is the total number of springs, $L_{c,j}$ is the contour length of WLC element j and is defined as 2.3 times the reference element length $L_{o,j}$, and r_j is the ratio of the instantaneous element length L_j to the contour length of spring j , i.e., $r_j = L_j/L_{c,j}$. The WLC-energy equation mentioned earlier differs from the conventional form as two constants c_1 and c_2 are introduced so that the spring elements have initial lengths. The constants c_1 and c_2 are set as 0.9673 and -0.9698 , respectively, from the definition of contour length [30].

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

Fig. 2 Illustration of the curvature of particle i . The shaded area is the area occupied by particle i , i.e. A_i . L_j is the length of edge j , n_1 and n_2 are the normal vectors to the two triangular elements which share edge j , and θ_j is the angle formed by these vectors, i.e., the included angle.

Fig. 3 Comparison of the geometry of (a) the initial discocyte with (b) the ellipsoid which is used to define the reference spring lengths, $L_{o,j}$, 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, surface area, and MAD of approximately $94 \mu\text{m}^3$, $134 \mu\text{m}^2$, and $0.332 \mu\text{m}^2$, respectively. The reference configuration is defined as follows: V_o is the reference volume with a value specified as the desired/target volume by the user, A_o is the reference surface area and in this work is specified as the initial area of the discocyte, ΔA_o is the reference MAD with a value specified as the desired/target MAD by the user, $C_{i,o}$ is the reference nodal curvature for node i with all nodes assigned the same value derived from ΔA_o , i.e., Eq. (7), and $L_{o,j}$ 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.,

$$E_{\text{Total}} = f(s_1 + s_2 + \dots + s_i) \quad (9)$$

where $s_i(x_i, y_i, z_i)$ is the position vector of particle i . Therefore, the internal force on a particle can be derived from the gradient of the model free energy [32], i.e.,

$$\mathbf{F}_i = -\nabla E_{\text{Total}}(s_i) \quad (10)$$

or

$$F_{i,x} = -\frac{\partial E_{\text{Total}}}{\partial x_i}, \quad F_{i,y} = -\frac{\partial E_{\text{Total}}}{\partial y_i}, \quad \text{and} \quad F_{i,z} = -\frac{\partial E_{\text{Total}}}{\partial z_i} \quad (11)$$

where $F_{i,x}$, $F_{i,y}$, and $F_{i,z}$ are the components of the internal force $\mathbf{F}_i$ 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

$$\Phi_i = \mathbf{F}_i \cdot d\mathbf{s}_i \quad (12)$$

where Φ_i is force potential and $d\mathbf{s}_i$ is the infinitesimal displacement. From the principle of energy conservation, the total force potential must equal the change of the model free energy, i.e.,

$$E_{\text{Total},n+1} = E_{\text{Total},n} - \sum_{i=1}^{N_p} (\Phi_{i,n}) = E_{\text{Total},n} - \sum_{i=1}^{N_p} (\mathbf{F}_{i,n} \cdot d\mathbf{s}_i) \quad (13)$$

where $E_{\text{Total},n+1}$ and $E_{\text{Total},n}$ 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 predicted by iteratively obtaining the internal force on each particle and incrementing each particle position by $d\mathbf{s}_i$ until for all nodes $|\mathbf{F}_i| < 10^{-21} \text{N}$. The incremental displacement $d\mathbf{s}_i$ is calculated as follows for each node:

$$d\mathbf{s}_i = \alpha \mathbf{F}_i \quad (14)$$

where α (10^6m/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., shearing 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

$$v = \frac{V_{\text{vesicle}}}{4/3\pi R_o^3} \quad (15)$$

where V_{vesicle} is the vesicle volume and R_o is the sphere radius equal to $3.265 \mu\text{m}$ for a surface area of $134 \mu\text{m}^2$. The nondimensional MAD is given by

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].

Reduced				
Vol.	MAD	Experimentally observed	Numerically predicted (4636 elements)	Numerically predicted (1604 elements)
0.60	0.53
0.60	0.65
0.60	0.95
0.60	1.1
0.86	1.1
0.85	1.2
0.84	1.2

$$\Delta a = \frac{\Delta A}{8\pi R_0 H} \quad (16)$$

calculated and, importantly, the calculation is independent of mesh density. 348 349

336 where H is the membrane thickness with a value of 4 nm. Note
337 that the initial vesicle configuration, therefore, has $v \approx 0.645$ and
338 $\Delta a \approx 1.01$ [34,35].

339 These predicted vesicle shapes were obtained using two differ-
340 ent meshes: a fine mesh with 4636 triangular elements and a
341 coarse mesh with 1604 elements. By observation, it can be seen
342 that the predicted transformed shapes using either mesh are very
343 similar to the observed vesicle shapes. Some differences are
344 observable with the coarse-mesh models, however, and are due to
345 the fact that the mesh elements are too large to correctly define
346 the curved surface. The close agreement with the observed shapes
347 indicates that the membrane bending energy is being correctly

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

Fig. 4 The numerically predicted Δa - v phase diagram of vesicle transformation. The predicted transformed shapes show contour maps of curvature. The boundaries shown are reproduced with permission from Seifert et al. [36].

taken as zero. They showed using the phase diagram that vesicle shape transformation was continuous and that large and small MADs tended to induce outward and inward undulations, respectively.

In Fig. 4, it can be seen that, in general, oblates are the dominant transformed shapes for reduced volumes and reduced MADs similar to the initial configuration. Oblates are stable in a narrow range of reduced MAD; a small decrease leads to a shape shift toward stomatocytes, while a small increase leads to a shift toward stretched oblates which are similar to flattened dumbbells. Any further increase of the reduced MAD leads to a shape shift that depends on the vesicle reduced volume, i.e., a shift toward dumbbells for vesicles with small reduced volumes and a shift toward pears for vesicles with large reduced volumes.

In these simulations, the predicted vesicle shapes are mainly affected by the membrane bending energy model. The very good agreement with the observed shapes and the agreement with the previously defined boundaries indicate the bending model is appropriate for use in calculating the membrane bending energy. However, it should be noted that a bending calculation is limited by any severely deformed mesh elements. When vesicles are severely deformed, some triangular elements tend to collapse to severely stretched, needle like elements which can lead to the overlapping of neighboring triangular elements. As a result, the prediction of transformed vesicle shapes is restricted to a maximum reduced MAD of 1.3 [36].

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 gives some results from this study showing predicted echinocytes III with a reduced volume $v \approx 0.645$ and a reduced MAD $\Delta a \approx 1.66$. 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 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 rims. 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 echinocytes 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 echinocyte 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 stomatocytes 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 invagination to form stomatocytes II, e.g., two undulations form in the stomatocytes. Eventually, these undulations merge to form a deep and elongated notch and form stomatocytes III.

It is very interesting to see that the transformed shapes in the SDE transformation are very similar to those in the vesicle transformation. First, oblates are the dominant shapes in a very narrow range of the reduced MAD around $\Delta a = 1.0$. 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., stomatocyte

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].

Shape Category	Reduced		Experimentally Observed	Numerically Predicted (SP-RBC)	Numerically Predicted (Continuum)
	Vol.	MAD			
Stomatocyte III	0.645	0.76
Stomatocyte II	0.645	0.83
Stomatocyte I	0.645	0.98
Discocyte	0.645	1.02
Echinocyte I	0.645	1.09
Echinocyte II	0.645	1.47
Echinocyte III	0.645	1.66

Fig. 5 Predicted echinocytes III with a reduced volume $v \approx 0.645$ and a reduced MAD $\Delta a \approx 1.66$. These transformed shapes were predicted using meshes with (a) 2664, (b) 4636, (c) 8610, and (d) 12,930 triangular elements.

Fig. 6 The numerically predicted Δa - v phase diagram of the SDE transformation from discocytes to echinocytes I, II, and III

Fig. 7 The numerically predicted Δa - v phase diagram of the SDE transformation from discocytes to stomatocytes I, II, and III

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 echinocytes, 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.

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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, m^2
c_1, c_2	worm-like-chain constants, (N, J)
C	curvature, m^{-1}
D	diameter, m
E	energy, J
$F, \mathbf{F}$	force, force vector, N
H	thickness, m
k_B	Boltzmann constant, 1.381×10^{-23} m ² kg/(s ² K)
k_B	bending rigidity, J
K_{LA}, K_{GA}	local, global area dilation moduli, J/m
K_{LB}, K_{GB}	local, global bending modulus, J
K_V	volume constraint modulus, J/m ²
L, L_c	length, contour length, m
N_P, N_S, N_T, N_W	number of particles, springs, triangles, springs sharing a particle
p	persistent length of worm-like-chain, m
r	stretch ratio, m/m
R	radius, m
$\mathbf{s}$	cartesian coordinate vector, m
T	absolute temperature, K
v	reduced/dimensionless volume, m ³ /m ³
V	volume, m ³
x, y, z	cartesian coordinate, m
Δa	reduced/dimensionless area difference, m ² /m ²

Greek Symbols

Φ	force potential, J
θ	angle, radian
μ	micron, 10^{-6}
ξ, ζ	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

Abbreviations widely used in text

ADE = area difference elasticity
MAD = membrane area difference
PM = plasma membrane
RBC = red blood cell
SDE = stomatocyte–discocyte–echinocyte
SK = skalak
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, ΔA , i.e.,

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

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

Fig. 8 A thin membrane element of area A . R_1 and R_2 are the principle radii of curvature at the neutral plane, and H is the element thickness.

Fig. 9 Calculation of the model bending energy using curvature. i is the particle index. For an edge j ξ_j and ζ_j are the surface-normal vectors to the neighboring elements, L_j is the edge length, and θ_j is the subtended angle.

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 θ_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

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

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_2 , 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

$$C = \frac{\theta L}{A} \quad (A6)$$

Fig. 10 Calculation of the model bending energy using the subtended angle. ξ and ζ are the surface normal vectors, L is the common-edge length, and θ is the subtended angle.

Therefore, the model bending energy can be calculated by substituting Eq. (A6) into Eq. (A5), i.e.,

$$E_B = \frac{K_B}{2} \left(\frac{(\theta - \theta_o)^2 L^2}{A} \right) \quad (A7)$$

where θ_o 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:

$$\cos(\theta - \theta_o) = 1 - \frac{1}{2!}(\theta - \theta_o)^2 + \frac{1}{4!}(\theta - \theta_o)^4 - \frac{1}{6!}(\theta - \theta_o)^6 + \dots + \frac{(-1)^n}{(2n)!}(\theta - \theta_o)^{2n} \quad (A8)$$

the $(\theta - \theta_o)^2$ term in Eq. (A8) can be replaced so that the bending energy can be written as

$$E_B = K_B \left(\frac{L^2}{A} \right) \left[1 - \cos(\theta - \theta_o) + \frac{1}{4!}(\theta - \theta_o)^4 - \frac{1}{6!}(\theta - \theta_o)^6 + \dots + \frac{(-1)^n}{(2n)!}(\theta - \theta_o)^{2n} \right] \quad (A9)$$

If both of the model elements are equilateral triangles and if all the element edge lengths remain constant during deformation, the bending energy can be further simplified to

$$E_B = K_B \left(\frac{L^2}{\frac{\sqrt{3}}{2} L^2} \right) \left[1 - \cos(\theta - \theta_o) + \frac{1}{4!}(\theta - \theta_o)^4 - \frac{1}{6!}(\theta - \theta_o)^6 + \dots + \frac{(-1)^n}{(2n)!}(\theta - \theta_o)^{2n} \right] \quad (A10)$$

or

$$E_B = k_B \left[1 - \cos(\theta - \theta_o) + \frac{1}{4!}(\theta - \theta_o)^4 - \frac{1}{6!}(\theta - \theta_o)^6 + \dots + \frac{(-1)^n}{(2n)!}(\theta - \theta_o)^{2n} \right] \quad (A11)$$

where $k_B = 2K_B/\sqrt{3}$.

Equation (27), without the fourth and higher-order terms, is the typical bending-energy model employed in conventional SP-RBC models [28,40], i.e.

$$E_B = k_B[1 - \cos(\theta - \theta_o)] \quad (A12)$$

From the derivation just above, it can be seen that this bending model is an approximation to the Helfrich bending model [41] with an error that is fourth order with respect to the angle difference between the instantaneous and reference configurations. Importantly, this bending model was formulated based on two assumptions. The first assumption is that one of the radii of curvature of the membrane is infinitely large and the second is that the model elements are nondeformable equilateral triangles. The first assumption means the model is not capable of accurately predicting the bending energy of surfaces with complex curvatures. In addition, the second assumption means the model cannot accurately predict the bending energy if the model undergoes deformation that leads to severely stretched triangular elements, as usually happens with severely deformed models.

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