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Erythrocyte Shape Simulation by Optimization of a Closed Fluid Lamina

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In our earlier work the morphology of red blood cells (RBC) was examined in terms of the mean mean curvature (MMC) of their cell membranes. A simulation of the different geometries of these shapes showed that the MMC increases from the sphero-stomatocyte to the sphero-echinocyte via the discocyte. We extend this work by using a free energy function based on curvature elasticity, curvature homogeneity and volume and area constraints in conjunction with two different simulation methods: a gradient relaxation method and a Metropolis Monte Carlo method based on importance sampling. The problem with conventional methods of simulation is that they lead to the trapping of the cell shape in a local minimum. Rather than processing the new shape after calculating each point on the surface, the new shape is only processed once every point on the old surface has been calculated. The RBC membrane is treated in the simulations as a single fluid lamina exhibiting viscoelastic characteristics. In the simulations the cell is assumed to have axial symmetry and it can therefore be described by a finite set of conic sections. We have so far been able to obtain energy minima corresponding to discocyte and stomatocyte shapes using a sphere as the initial configuration. Similar shapes are to be found in volume deficient phospholipid vesicles. Our results which are restricted to the region of axial symmetry imply that this part of the well-known sequence of RBC shapes could indeed be governed by the properties of an ideal fluid forming a closed single connected sheet. Extensions to arbitrary shapes in three dimensions are planned with a view to simulating the echinocyte.

Keywords: Erythrocyte shape, membrane model, optimization, fluid lamina, curvature homogeneity

INTRODUCTION

The main purpose of the work reported here is the computer simulation of the morphology of red blood cells (RBC). This should lead to a better understanding of the physical laws governing the properties of the whole cell membrane in relation to the known molecular properties of membranes. The basis of this work is therefore the modelling of cell shapes in terms of fluid lamina representation of the membrane. In all the calculations presented here we restrict ourselves to equilibrium cell shapes.

Figure 1 shows typical erythrocyte shapes and includes discocytes and stomatocytes which are axially symmetric, sphero-echinocytes and sphero-stomatocytes showing local symmetry and echinocytes which exhibit a more complex symmetry. The various RBC shapes are characterised by the mean mean curvature (MMC). The MMC is one of the basic parameters which relates our theoretical work to experimental results and it can be regarded as a global shape parameter.[3] The geometrical interpretation of the mean curvature and the associated MMC is given for the discocyte shape in Figure 2. This figure shows the region of the two dimples which exhibit negative local mean curvature, whereas the rim region has a positive mean curvature. The section connecting these two regions is a saddle surface which can have both negative and positive mean curvatures.

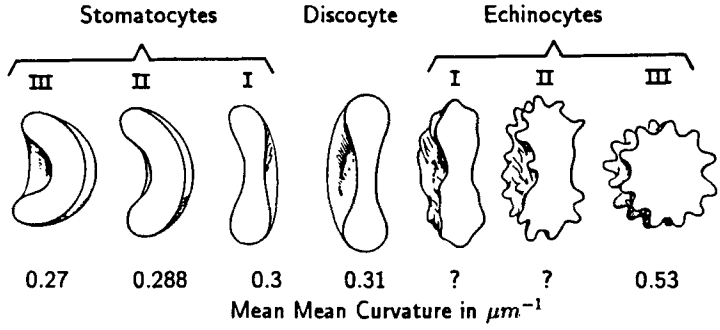

FIGURE 1 The sequence of typical erythrocyte shapes as given in the work of Bessis.[2] The Roman numerals refer to the usual classification whereas the numerical values give the MMC as found in our earlier work.

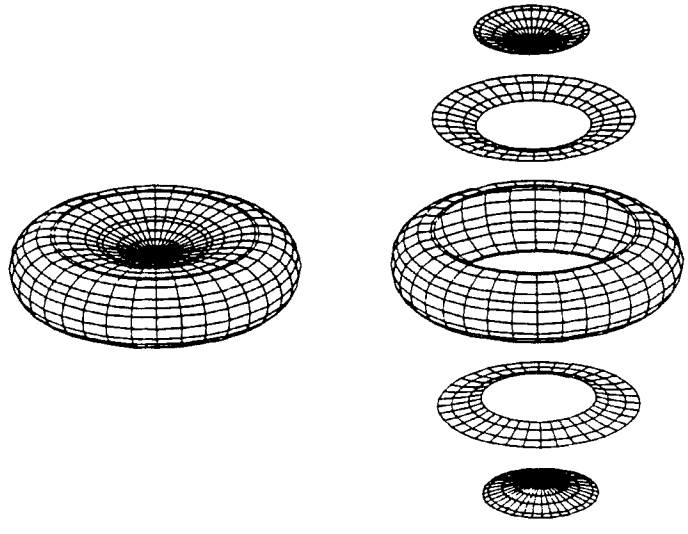

FIGURE 2 Subsurfaces of a discocyte. The rim region has positive mean curvature, the two dimples exhibit local negative and the intermediate section has both negative and positive mean curvature (modified after Reference 3).

In Figure 1 we examined the morphology of the RBC in terms of the MMC by using prefabricated cell sections similar to the methods used by other authors e.g. in References 1 and 8. The problem with this procedure is that the matching of the subsurfaces introduces discontinuities in the mean curvature at the lines of contact which in their turn introduce ambiguities in the results of any optimization procedure. To overcome this disadvantage several authors used analytic descriptions such as the Cassini equations or polynomials, but this immediately reduces the number of possible configurations.

FREE ENERGY AND OPTIMIZATION METHODS

Our aim is to simulate axially symmetric RBC shapes ab initio using both deterministic and stochastic optimization procedures. The purpose of the optimization procedures is to minimize the total free energy of the whole membrane. In our model, the free energy function for the closed fluid lamina has the following contributions:

- the compression elasticity of the lamina,

$$E_A = K_A \frac{(A_0 - A)^2}{A_0} \tag{1}$$

- a term depending on the volume enclosed by the lamina which is related to the osmotic pressure across the membrane,

$$E_V = K_V \frac{(V_0 - V)^2}{V_0} \tag{2}$$

- an averaged curvature-elastic energy which depends only on mean mean curvature.

$$E_{\overline{c_m}} = K_B \frac{(\overline{c_{m0}} - \overline{c_m})^2}{\overline{c_{m0}}} A \tag{3}$$

A novel feature of the simulation used in this paper is the inclusion of a quadratic term in the local curvature which determines the final equilibrium shape:

$$E_{c_m} = \frac{K_c}{A} \int_S \left(\frac{d\, c_m(l)}{d\, l} \right)^2 ds \tag{4}$$

where l is given in Figure 3. This term limits the fluctuations of local mean curvatures, $c_m(l)$, by minimizing differences in neighbouring areas (lamina densities), thereby controlling curvature homogeneity. This concept is explained in Figure 3. The total free energy is obtained by summing the terms given by Equations (1) to (4).

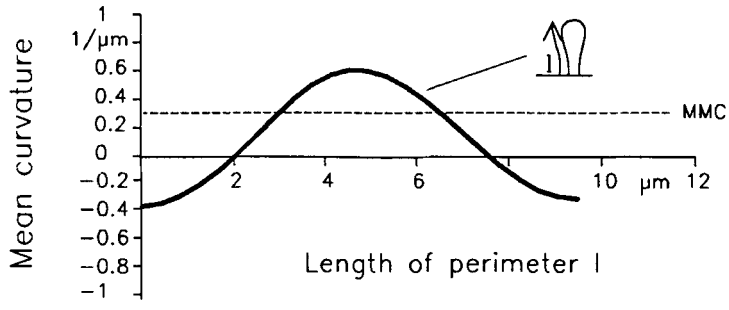

FIGURE 3 Local mean curvatures for a discocyte as function of the perimeter l of the cross-section of the solid of revolution.

We have successfully used two simulation methods for the numerical optimization of the single fluid lamina model. These are:

- a multidimensional gradient method,
- a Monte Carlo method using a modified Metropolis algorithm.

The details of the simulation methods are summarized as follows:

1. The first method, which is also known as gradient relaxation, is a deterministic method based on Newton's equations, which are used here to describe the forces on surface points and the resultant displacements. The algorithm for this method can be implemented in the following manner. In the case of our fluid lamina model, an initial configuration (in our case a sphere) is chosen for the system. The vertices of the polygon describing the surface are then visited sequentially and the vertex under consideration is first moved to the perpendicular bisector of the line joining the neighbouring vertices as shown in Figure 4a. Next as also shown in Figure 4b the force acting on the related surface section is determined by means of a partial relaxation of the vertex. The usual method is then to accept the move and continue to the next vertex. However the observation that the system could easily fall into one of many local free energy minima implies that a different procedure is required to prevent the system from being trapped in such a minimum. In our case we use a novel method which is suited to the simulation of cellular shapes. All points on the surface are moved relative to the previous configuration according to the deterministic algorithm. The displacement vectores are stored without changing the old configuration. After every point on the surface has been treated, the new cellular configuration is modified by the displacement vectors times the energy decrement factor and is then accepted as a whole. A new cell shape is thus generated at each pass over the system. Additional random noise due to the temperature of the system can be added to each vertex movement independently so as to reach an equilibrium configuration.
2. The standard Metropolis Monte Carlo method[7] is a stochastic method which generates new configurations of a given system by means of an energy criterion. Usually a change in internal degrees of freedom is made locally, the difference in energy is calculated and the criterion which accepts the change

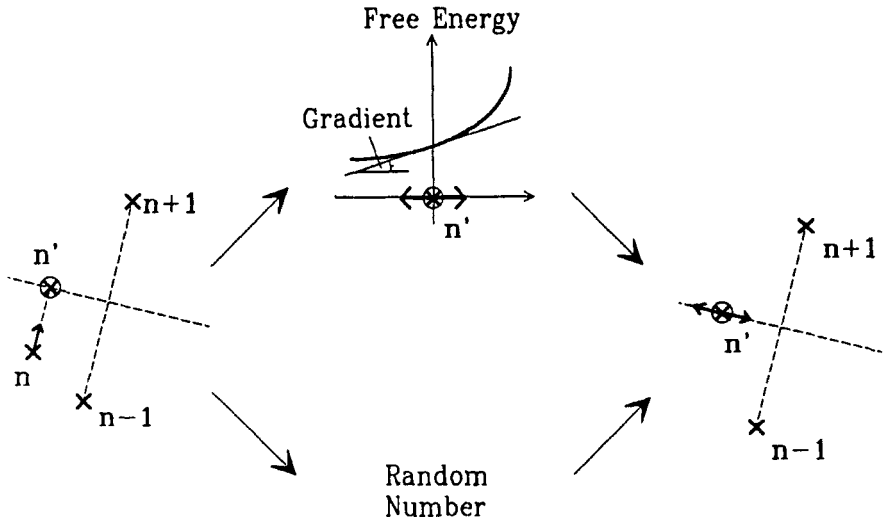

FIGURE 4 Optimization procedure (from left to right): vertex X_n is moved to the perpendicular bisector of the line joining X_{n-1} X_{n+1}; the displacement is determined by the gradient of the total free energy (deterministic method) or the standard Metropolis Method; all vertices are moved simultaneously along the perpendicular bisector with respect to their nearest neighbors.

is such that the system approaches equilibrium at a fixed temperature by minimization of the free energy. The method is well documented and the reader is referred to standard texts on the subject. An initial configuration (as above) is chosen for the system. Next, a point on the surface is selected randomly and moved a random distance perpendicular to the surface, the distance being less than a maximum value which is a fraction of the displacement between surface points. The details of the move are explicitly described in Figure 4. In the standard Metroplis method, the energy criterion is used to accept or reject the move, a new configuration is generated and the simulation continues at another randomly selected point. The approach to equilibrium is easy to observe for well-behaved systems with a single free energy minimum. However, as mentioned above, the free energy has many local minima, in which the system can be frozen during the simulation. This problem has been partially resolved by Kirkpatrick *et al.*[6] who proposed an optimization method known as simulated annealing, in which the system is slowly cooled to its equilibrium state.

The stochastic method used in our simulations is based on the standard Metropolis Monte Carlo method for fluids using importance sampling. However, in contrast to the implementation of the usual algorithm, the system is updated at the end of every Monte Carlo pass over the entire system rather then after every individual Monte Carlo step, as was also the case for the deterministic method.

An advantageous feature of both optimization methods is that each method allows an occasional free energy increase between configurations, implying that the system

is able to "jump" out of a local energy minimum. This is because the non-linear behaviour of the energy between individual configuration changes is equivalent to a sampling of phase space over many local energy minima.

RESULTS

Figure 5 shows the free energy as a function of time (number of passes) as found from the gradient relaxation method after an incremental change in one of the constraints. This figure shows the first 160 passes of a typical optimization run after an incremental change in cell volume from 144 μm^3 to 94 μm^3 while keeping surface area and mean mean curvature constant. It can be seen that the system falls into and jumps out of different local energy minima. The overall energy initially decreases and eventually saturates. The minimum with the lowest energy value is taken as the global minimum.

Figure 6 shows three cell shapes corresponding to different energy minima. The first two shapes correspond to intermediate energy minima of Figure 5 and exhibit a large variation in local mean curvature. The third shape which is discocytic and represents the global minimum has a much smaller variation in local curvature. Each shape has the same volume (94 μm^3), the same surface area (134 μm^2), and the same mean mean curvature (0.317 μm^{-1}). The difference of the local mean curvature between neighbouring areas is the source for the differences in local composition and is directly related to the chemical free energy density of the membrane. Figure 6 shows the progression towards curvature homogeneity during an optimization procedure using gradient relaxation.

In order to confirm the hypothesis concerning MMC postulated in our earlier work,[3] we used the gradient relaxation method to model a progression of typical axially symmetric RBC shapes as a function of MMC. The initial value of the MMC (0.32 μm^{-1}) was that of a discocyte and the other values were for stomatocytes with increasing stomatocytosis. In the simulation the MMC was decreased in small steps (0.01 μm^{-1}). For each new step the optimization procedure was carried out

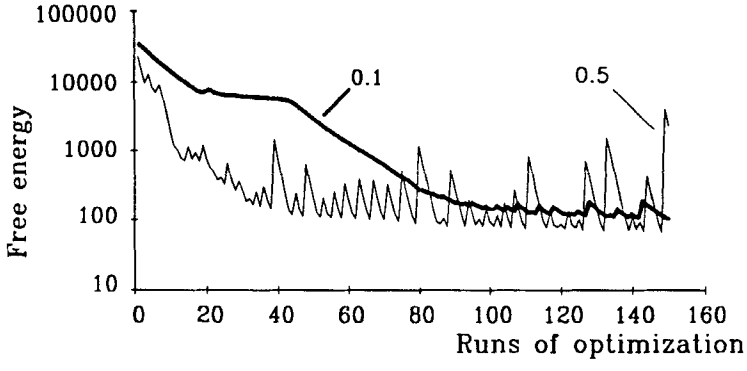

FIGURE 5 Free energy as a function of the number of optimization steps (passes over the system) after an incremental change in one of the constraints for two different energy decrement factors (0.1 and 0.5).

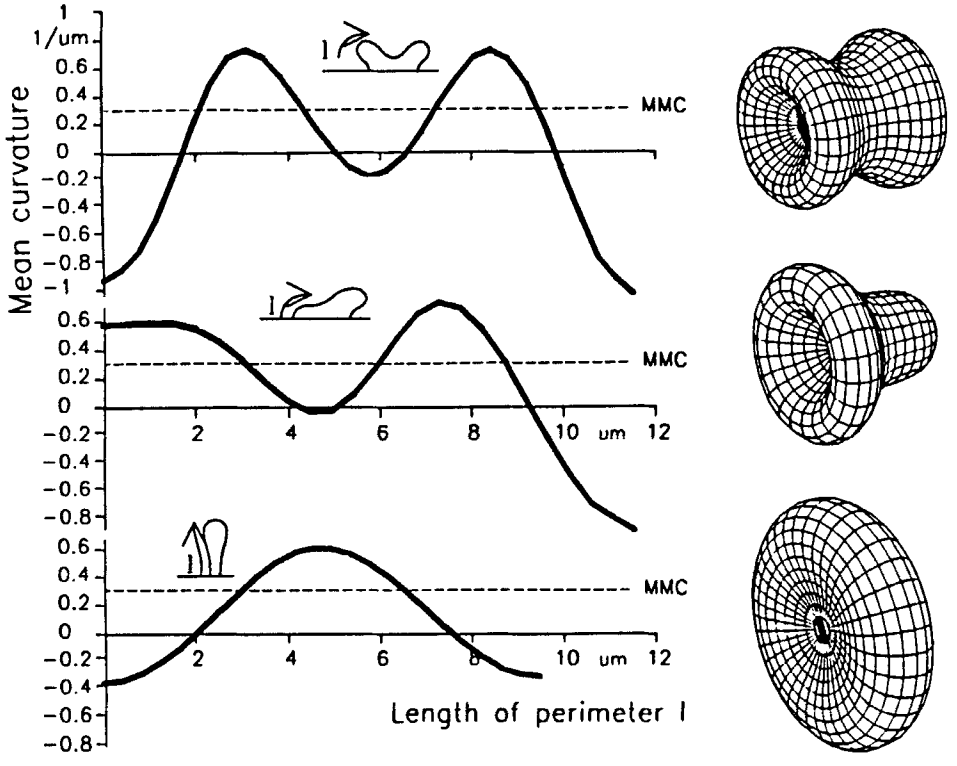

FIGURE 6 RHS: Shapes exhibiting local free energy minima (upper figure) and the probable global one (lower figure) obtained during optimization with constrained values of volume 94 μm^3, surface area 134 μm^2, and MMC 0.317 μm^{-1}. LHS: Related crossections of the solids of revolution and the local mean curvatures as function of the perimeter l.

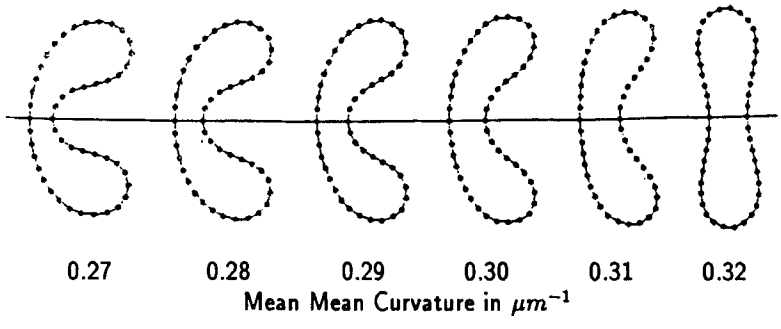

FIGURE 7 Sequence of RBC-shapes obtained by stepwise optimization of the previous configuration after decrease of MMC.

by starting from the previous configuration. The configuration representing the new global minimum was then determined and the relevant parameters were calculated. The resulting sequence of configurations is shown in Figure 7. It exhibits a behaviour which is found in data obtained from shape altering experiments with

erythrocytes. Identical results are obtained using the Monte Carlo method described in the previous section.

CONCLUSION

We have shown that numerical optimization based on the fluid lamina model using the global properties of constant volume, area and mean mean curvature, and the optimal homogeneity of local mean curvatures leads to the commonly observed erythrocyte shapes. Compared with former membrane models the advantage of this model is that it requires no assumptions apart from the well established mobility of the membrane components. The local viscosity implies a global elasticity due to both the finite size of the cell and the area constraint. Our model is therefore completely consistent with the fluid mosaic model.

Evans (private communication) has suggested that the presence of local energy minima in our simulation results may be due to the use of a polygonal approximation for the cellular cross-section in the simulations. We intend to check this by greatly increasing the number of verticies used in the simulation. Another point is that the curvature-elastic free energy has been included in the free energy function as an average as is appropriate for a fluid lamina to give a global elasticity. We intend to investigate the effects of including the full curvature elasticity by going continuously from our expression to Helfrich's well-known expression in terms of the local curvature[5] by means of a scaling factor.

We have performed several additional simulations which will be reported elsewhere. These include:

- Change of shape under micro-pipette aspiration.
- Endo- and exo-vesiculation for extreme values of the MMC.
- Confirmatory simulations using the method of systolic annealing in conjunction with transputers.

We are in the process of setting up three-dimensional simulations for RBC shapes without and with spectrin.

Acknowledgments

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