Simulation of Erythrocyte's Deformation Using Conformational Changes in the Cytoskeleton

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Abstract - Erythrocytes demonstrate an ability to deform considerably and move through capillaries which are up to six times smaller than their unperturbed diameter. It is suspected that this deformation is not purely elastic. A discrete model for the mechanic response is proposed, based on a mechanism which allows the erythrocyte's cytoskeleton to disconnect and reattach interconnecting proteins. The model explains the known dependency of the erythrocytes' elasticity on ATP levels, and the theoretical predictions can be tested by suggested experiments.

Key-Words: Mechanics, Cytoskeleton, Deformation, Erythrocyte, Simulation, Spectrin, ATP.

1. Introduction

1.1. Erythrocyte membrane's structure

The erythrocyte's cytoskeleton is shaped as a two dimensional net, attached to the inner side of the cell membrane's lipid bilayer. The major protein constituent of the membrane skeleton, spectrin, accounts for 75% of the skeletal mass [16]. Spectrin are filaments (~70-100nm) that crosslink short actin nodes (~30nm), and connect to the lipid bilayer through the protein ankyrin.

A two dimensional grid is created by spectrin filaments that connect junctional complexes, composed of actin and other proteins [2,27]. Each junctional complex is connected to five to seven other complexes, thus creating a mesh made of equilateral triangles (fig.1). In a human erythrocyte there are approximately 10^5 spectrin filaments interconnecting $3*10^4$ junctional complexes [6].

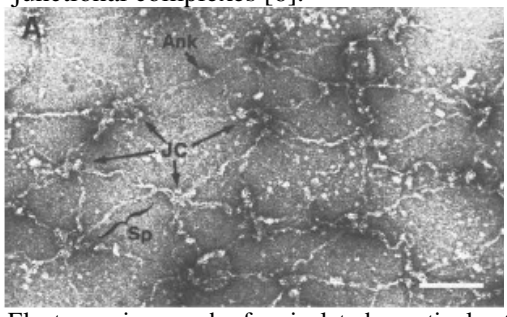


Fig.1, Electron micrograph of an isolated negatively stained RBC membrane skeleton of adult mice, showing the spectrin mesh. [21]. Sp, spectrin filament; JC, junctional complex, Ank, ankyrin.

1.2. ATP-induced Spectrin-actin dissociation

Recent studies have shown that adenosine triphosphate (ATP) can dissociate the covalent bonding between the spectrin and the actin at the junctional complexes, and thus induce structural changes [8]. Dissociating spectrin from actin requires ~$5k_bT$, while hydrolysis of ATP releases ~$13k_bT$ [2]. The excess in energy means that the dissociated spectrin filament can change its orientation and reattach to other actin nodes [8], overcoming energetic barriers due to steric repulsion.

2. Simulating Conformational Changes

2.1. Constitutive elements

The presented model simulates the erythrocyte cytoskeleton's mechanical response to deformations up to 600 percent, focusing on the conformational changes of the spectrin-actin grid caused by the dissociations of the spectrin filaments.

We examine a small patch of the net, in the order of hundreds of junctional complexes (~1% of the total number of junctional complexes in human erythrocyte). Spectrin filaments are modeled as one-dimensional linear springs, with a free length L_0, and a spring constant k_{sp}. Assuming elastic behaviour, the force a filament exerts on the actin nodes, F, is: (1) $F = k_{sp}(L - L_0)$, and the elastic energy it stores, E, is: (2) $E_e = k_{sp}(L - L_0)^2$. When compressed, we assume that the spectrin filaments apply negligible

forces, since they can bulge out of the two dimensional plane.

It should be noted that experiments using atomic force microscopy showed that spectrin monomers in vitro divert from linear behavior, due to unfolding of the spectrin [28]. However, atomic force microscopy invloves stretching the spectrin up to its full contour length, which is three time as long as its free length in the cytoskeleton [16,17]. Therefore, in vivo elastic characteristics of the spectrin remain unclear, yet even if k_{sp} is not constant or linear, the behavior of the model will not change qualitatively, as it is based upon minimum energy solutions, which correlate to minimum elongation.

The junctional complexes are modeled as dimensionless points, which connect to six other junctional complexes, in the free state, to create the triangular grid (fig.2). To compensate for the dimensionless modeling, strong steric forces are present if the junctional complexes are drawing close.

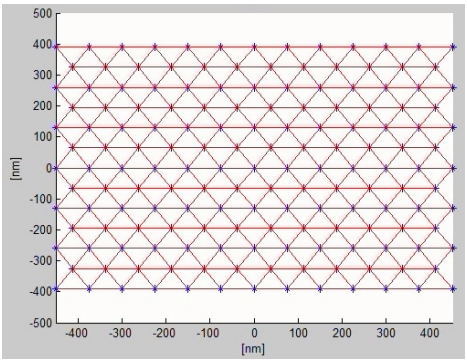


Fig.2, Initial (unstrained) grid, containing 163 junctional complexes and 438 spectrin filaments.

2.2. **Model deformation**

The model's dynamic response under strain is based on three physical attributes of the cell: constant volume, inner equilibrium and frequent spectrin dissociations. Constant volume is in agreement with experimental data [19], and moreover, hydrostatic volume change is orders of magnitude smaller than the strain amplitude. Inner equilibrium means that the junctional complexes should reach equilibrium under the forces which act upon them, including the elastic forces of the spectrin filaments, the steric repulsion from other junctional complexes and the restricting friction of the lipid bilayer. Frequent spectrin dissociations means that the process of dissociating a spectrin filament from a junctional complex, and reassociating it to the same or a different junctional complex, is occurring in multitude during the time needed for the deformation process. This attribute is based on the short time needed for the reassociation of spectrin: $\tau_{re} \sim 10^{-7}$ s [10,14], and the fact that the observed effects of ATP on the membrane reaches saturation at low ATP concentrations [31] (i.e. The dissociations are frequent enough, even in low ATP concentrations, that adding ATP to induce more dissociations have only slight effects).

Figure 3 demonstrates the underlying process by which the reassociation of spectrin filaments, catalyzed by ATP, can initiate a change of form in a strained cytoskeleton. Following the process in figure 3b, two actin nodes are connected to five spectrin filaments, and two are connected to seven, which makes the spectrin-actin connections less stable, and help to propagate the change in the entire grid.

The initial form of the grid is approximately square, and in the deformation process we gradually move the junctional complexes further apart in the horizontal axis, while drawing them closer in the vertical axis. This is done while maintaining constant area by applying appropriate boundary conditions. The straining is taken as uniform expansion and therefore applied to all the junctional complexes simultaneously. The simulation includes successive two-steps of straining and relaxation in the following order:

A. Straining: A fraction of the complete strain is applied through forced movements of junctional complexes.

B. Relaxation:

- Spectrin filaments are rearranged to reach a minimum energy conformation, where their elastic energy is minimal, and therefore the filaments are most stable.
- The forces on the junctional complexes are calculated and those without boundry conditions are moved towards their equilibrium point.
- Phase B is performed for a predetermined number of repeated steps.

C. The process is repeated until complete strain is reached.

During the model's deformation process, spectrin filaments are allowed to cross one another in order to find the minimum energy solution. While this state is less stable, it could occur as a transition state due to the fact that actin nodes which are connected to only five spectrin filaments tend to buckle out of the local plane [8], allowing the crossing over. Furthermore, as

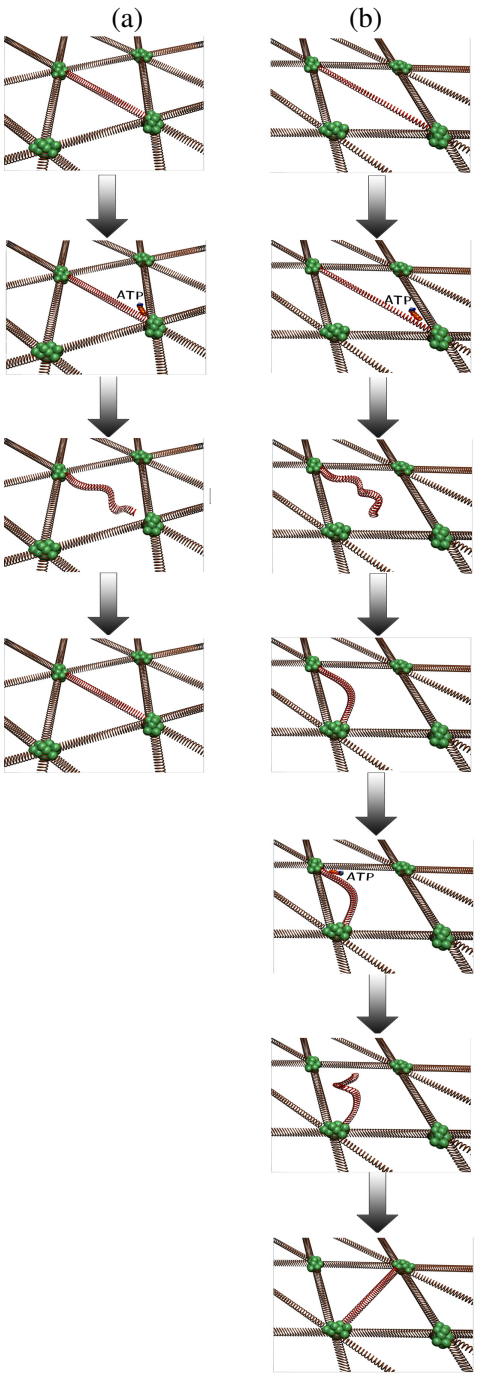


Fig.3, A sketch of the spectrin dissociation and reassociation process in (a) an unburdened grid and (b) in a strained grid. In the burdened state, a lower-energy configuration is found for the elongated spectrin filament.

the strain on the erythrocyte increases, so does the three dimensional curvature of the grid, meaning that the overlapping spectrin filaments are on different planes and do not intersect.

2.3. **Model parameters**

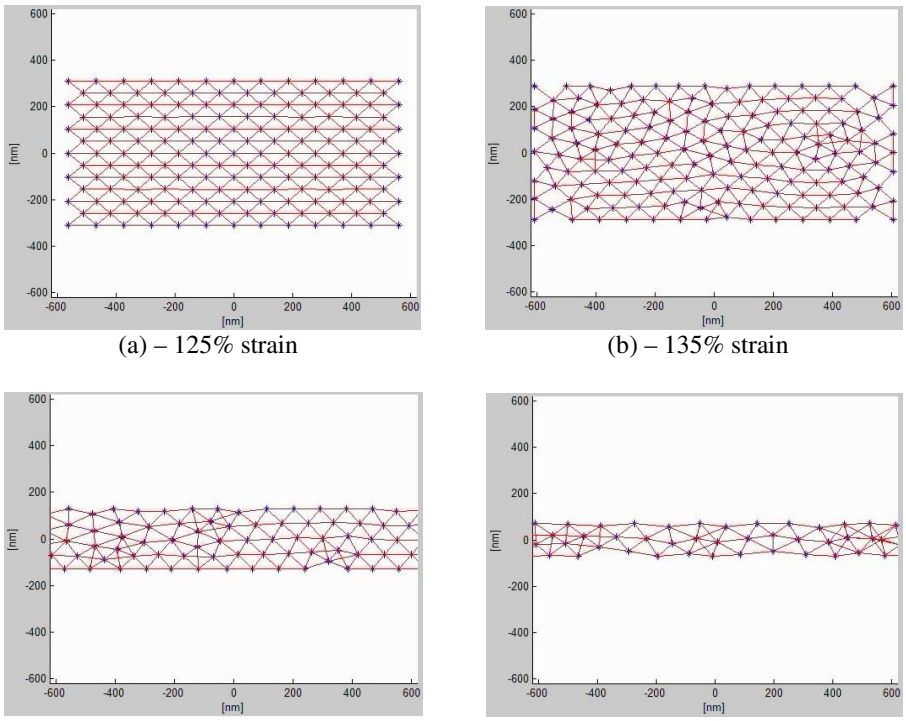
By preventing from the junctional nodes to reach their equilibrium points between deformation steps, we essentially allow the rate of deformation to become a factor, so it is possible to simulate different paces of deformation. The rate is represented by the ratio of strain increments to relaxation cycles: a fast deformation process, for example, is characterized by large strain increments with few relaxation cycles.

Other model parameters relate to the biological characteristics of the erythrocyte. The only known relevant characteristic of the cytoskeleton, which we can incorporate into the model, is the distance between junctional complexes, $L_{initial}$~75nm [22]. We can also assume that the free length of the spectrin filaments, L_0, is of the same magnitude. The unknown biological parameters include the spring constant of the spectrin filament, k_{sp}, the strength of the steric repulsion between the junctional complexes and the exact ratio of spectrin filaments to actin nodes (it is known that there are roughly three filaments per actin node).

3. Results

The ability to reconnect spectrin filaments in lower energy configurations have allowed the grid to respond dynamically to the strain. Due to geometric characteristics the response remains purely elastic up to ~130% strain (fig.4a), whereas under larger strains, minimum energy solutions are found through a mechanism of reattaching the interconnecting proteins in new formations (fig.4b). This process leads to stable formations even under very high strains (fig.4c,d), as almost any shape can be roughly divided into equilateral triangles through the mechanism.

The conformational changes initiate at ~130% strain due to geometric reason: vertically adjacent points in neighboring equilateral triangles become closer than horizontally adjacent points (similar to fig.3) at 131.6% strain. This characteristic creates an “energy barrier”, after which the elastic energy stored in the grid drops (fig.5). In vivo, it could be assumed that the change is less abrupt since the grid is not evenly divided, yet the “energy barrier” would still be present since this is an equilateral triangle’s geometric characteristic and does not depend on any of the parameters. As the strain continues to grow, the elastic energy begins to slowly rise again, despite the fact that any form of the grid could be roughly divided into equilateral triangles. This rise is caused by disallowing the grid to reach equilibrium between straining cycles.



(a) – 125% strain (b) – 135% strain

(c) - 300% strain (d) – 550% strain

fig.4, An example of the model deformation up to 550%, with the following parameters: L_0=77.25nm (=1.02*$L_{initial}$), number of spectrin filaments to actin nodes = 3, steric repulsion = 2·K_{sp}, deformation speed = 4 relaxation cycles per percent strain. Note that the axes are set, and therefore after 135% strain, the grid elongates beyond the image's scope and only the central portion is displayed here.

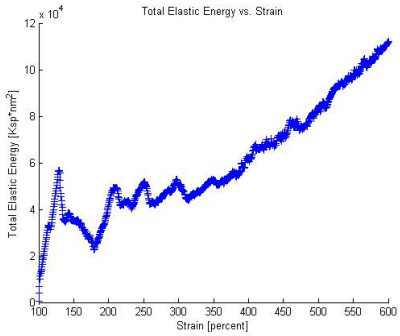


Fig.5,The elastic energy present in the entire grid, under the same parameters as in figure 4. The elastic energy includes both the energy stored in the spectrin filaments, and the energy derived from the steric repulsion of the actin nodes.

Also, "nucleation areas" are formed, where several actin nodes are clustered in a small area, held together by numerous spectrin filaments against the steric repulsion, creating strain in the area and a shape which cannot be evenly divided.

Another effect of the reassociation model is that the spectrin filaments do not elongate proportionally to the strain applied (fig.6), and even under 600% deformation, the average elongation is ~20% and the most elongated filament reaches ~60%. As in the elastic energy, initially the filaments elongate proportionally, but as lower energy solutions present themselves, the rapid elongation process halts. Hence, the simulation leads to the conclusion that most of the global elongation is achieved by local realignments. It should be stressed that due to the discrete reassociations, this behavior cannot be described by classical continuum theory, since neighbor elements do not remain neighbors.

Yet another characteristic of the model is that when presented with defects in the cytoskeleton, it showed an ability to return to a stable conformation. Defects of the nature of a movement of several actin nodes, or the addition of an actin node required few relaxation cycles to allow the grid to recreate the form of equilateral triangles. However, if an actin node was removed, the subsequent hole in the grid could have been bridged over only by applying strain to the grid.

The model also tested the effects of the various parameters: Abundance of spectrin filaments (>3 filaments per actin node) led to rigidity and eventually to shearing of the grid under high strain. Lack of spectrin filaments also led to shearing of the grid, due to forming of holes in the grid, and their propagation

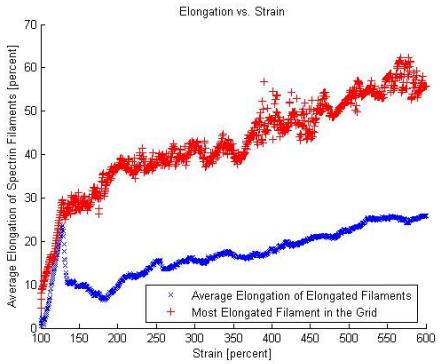


Figure 6. Elongation of the spectrin filaments [%]. Showing the most elongated filament in the grid, as well as the average elongation of the elongated filaments (discarding compressed filaments).

under strain. The strength of the steric repulsion between the junctional complexes was needed to be high enough to prevent "nucleation areas" (see above). The model runs were made using a steric repulsion spring constant equal to $2 \cdot k_{sp}$, which acted when junctional complexes were closer than $L_{initial}$. The equilibrium length of the spectrin filaments, L_0, was found that if shorter than $L_{initial}$ the grid is constantly under tension, and raptures under high strains. For $L_0 > L_{initial}$, the grid remains stable throughout the process, though as the difference increases, so does the instability of the grid in the free state since the forces that hold together the actin nodes are weaker.

4. Discussion

A recent computer simulation performed on a macro scale[15], which included spectrin rearrangement yet not the forces of the spectrin on the junctional complexes, have shown that the cytoskeleton macro-structure can remain stable in a biconcave shape, and in deformations of up to 200%. The presented model, focusing on the micro scale, portraits the mechanics of the conformational changes of up to 600% strain, and leads to further conclusions.

The model showed that an energy barrier is present at approximately 130% strain, meaning that the change in the grid of the cytoskeleton will not be initiated under any force or deformation. This supports the findings that the erythrocyte's shape affects its oxygen transferring characteristics [32], and accordingly should not be initiated by inconsequential forces. However, once the energy barrier has been overcome, elastic energy rises significantly slower and the cytoskeleton remains stable.

Another noteworthy result of the model is that the number of intersecting spectrin filaments rises with the strain. As explained in section 2.2, as the strain rises so does the three dimensional curvature of the grid, hence the filaments do not cross, but overlap each other. Their propagation with the strain stabilizes the bending of the erythrocyte and might explain the folded shape it receives when in capillaries. It can also explain the mechanism by which the erythrocyte returns to its original shape, since these connections are unstable when the erythrocyte unfolds, and the cytoskeleton's curvature decreases.

The model also gives an explanation to the mechanism by which aged erythrocytes die in capillaries. ATP concentration in the cells decrease as the cell matures [23], or as it nears the end of its life span [29], which impedes the dissociations of spectrin filaments and prevents the rearrangement of the actin nodes. Under this condition, the grid would not be able to find lower energy configurations and the elastic energy in the grid, as well as the elongation of the spectrin filaments, will rise steeply (fig.7). These energies might lead to unfolding of the spectrin filaments and damage the actin nodes or the membrane.

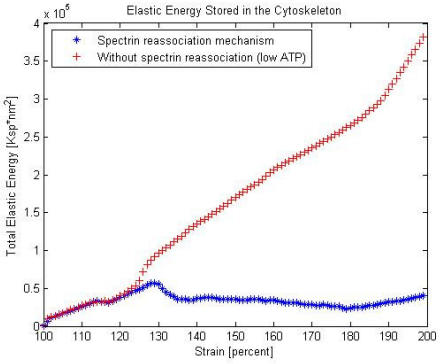


fig.7, Comparison of the elastic energy present in the model, with and without spectrin reassociation, up to 200% strain.

We would like to suggest two methods of verifying the model. The first consists of fluorescenting a few spectrin filaments and actin nodes in an erythrocyte, and measuring their relative distances, before and after undergoing a straining process, such as entering a micropipette. According to the model, the relative distances should change after the straining process, signifying that the actin nodes and the spectrin filaments altered their respective positions. Another possible method is to filter erythrocytes for a certain diameter, and measure the energy needed, for those erythrocytes, to enter micropipettes of varying sizes. According to the model, the gradient of the energy should change considerably when the diameter of the

micropipette is 125%-135% narrower than the diameter of the erythrocytes.

In conclusion, the model demonstrates how the erythrocyte's cytoskeleton can endure large deformations via changes to its structure, based on changing the connections between any two actin nodes to reach minimum energy conformations. While the model undoubtedly simplifies the process and other factors are involved, it underlines how the known characteristic of disconnection and reattachment of spectrin filaments can be used to explain a unique method of withstanding large strains.

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