Characterization of Red Blood Cell Damage Under Large Deformation

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Abstract

The behavior of red blood cell (RBCs) under high deformations and mechanical stresses is of great interest in many biomedical applications, such as in artificial hearts, hemodialysis machines, and ventricular assist devices. Under such high stresses, RBCs might experience sub-lytic damage in the form of temporary pore formation and hemoglobin release, or in more critical cases, complete rupture and lysis. This study utilizes a two-component coarse-grained molecular dynamics (CGMD) model and experimental methods to study the pore formation and cytoskeleton deformations during squeezing through a microfluidic channel. The focus is to find the pore formation criteria and the interaction of the cytoskeleton with the lipid bilayer and pores.

Motivation and Goals

- Continuum models of RBC damage are device-dependent and do not incorporate the physical process of pore formation.
- We aim to develop the first cellular model for red blood cell damage and hemolysis assessment under clinically relevant flow condition.
- Bridge three different scales to understand underlying physics of the problem.
- Propose a universal cellular scale design criteria to prevent the danger of Hemolysis by studying red blood cells damage as a result of high mechanical stresses.

Molecular Dynamics Simulation

A two-component particle based CGMD model is employed to explicitly represent lipid bilayer and cytoskeleton particles. Spherical particles with both translational and rotational degrees of freedom are used to represent lipid particles. The cytoskeleton consists of spectrin tetramers tethered to the lipid bilayer at the actin and band-3 junctional complexes. The spectrin tetramer is modeled with 39 beads connected to each other with unbreakable harmonic bonds. They are also connected to the immobile band-3 proteins. The junction units that connect the lipid bilayer to the cytoskeleton are modeled by glycophorin and actin particles which are bonded to each other using unbreakable springs. Lennard-Jones potential is used to model the actin and spectrin interactions with the lipid bilayer. To decrease the computational cost of the simulations, the solvent is represented implicitly, and lubrication forces are neglected. The Langevin equation governs the translational motions of CGMD particles while velocity-verlet algorithm with the timestep of $\Delta t=0.01\tau$ is used to integrate the motion equations.

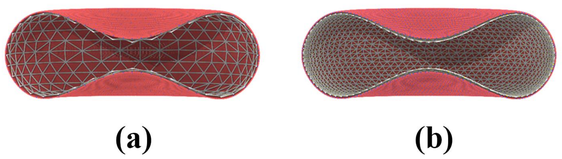

Figure 1. (a) CGMD one particle thick RBC model with 512 vertex points; (b) with 3052 vertex points;

Squeezing Simulation of RBC

Squeezing through narrow constriction can generate strain in the RBC membrane and subsequent pores. CGMD simulation of squeezing RBC through narrow constriction of different geometries (Changes in width and length) reveals critical constriction parameters for a RBC for squeezing through, clogging and vesiculating.

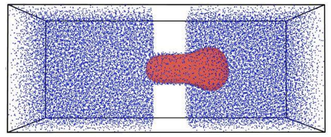

Figure 2 .Constriction width= 20σ, length= 20σ, Push force=0.01

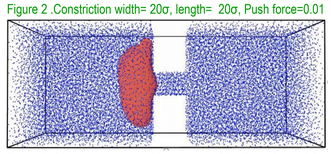

Figure 3 .Constriction width= 10σ, length= 20σ, Push force=0.01

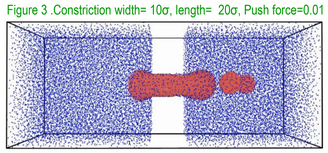

Figure 4 .Constriction width= 10σ, length= 20σ, Push force=0.02

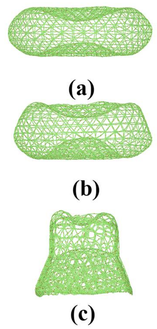

Figure 5 .Distribution of cytoskeleton network (a) before, (b) at the entrance (c) after entering the narrow constriction

For the same constriction parameters, increasing the pressure leads to vesiculation in the RBC membrane. The squeezing process deforms and redistributes the cytoskeleton network with increasing density of network at the tail of the RBC.

Squeezing Experiment of RBC

Experiment of RBC squeezing through microchannel has been done in a 2μm wide and 20μm long channel with 40μl/min flow rate. Quantitative Absorption Cytometry (QAC) was used to measure the drop of hemoglobin in RBC during squeezing.

Figure 6 .Absorption imaging of RBC in a narrow channel.

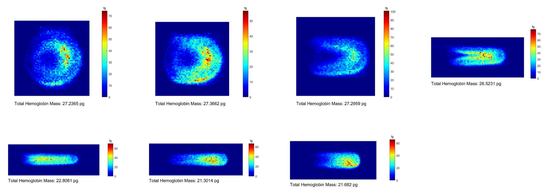

Figure 7 .Hemoglobin mass mapping of an RBC squeezing through a narrow constriction

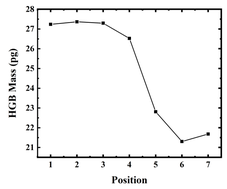

Figure 8 .Total mass of hemoglobin vs position in narrow channel for a single RBC

A decrease of approximately 5pg in total hemoglobin mass was observed while the RBC was squeezed through the narrow channel.

One-Particle-Thick Model Limitations

The physical process of pore formation require the hydrophilic end of bilayer membrane to seal the hydrophobic end at the pore opening. The one-particle thick model does not have hydrophilic and hydrophobic poles. The membrane is modeled with ellipsoidal particles instead, which have particular direction for stability. The particles are only stable when their directions are parallel to each other. For this reason, the pore formation in one particle thick model cannot be considered accurate.

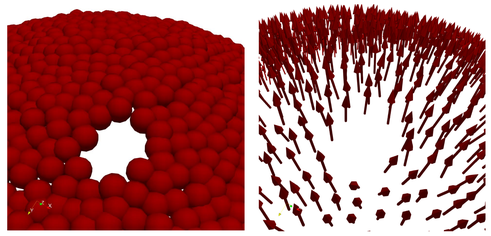

Figure 9 . Pore formation due to swelling in one particle thick model and subsequent vector direction of the ellipsoids. No stable hydrophilic pores can be captured by one-particle-thick models

Head-Tail Model

In order to avoid the limitation of one particle thick model, finer head-tail models such as that of Cooke, Kremer and Deserno can be utilized to capture hydrophilic pores. This model has been modified with the inclusion of cytoskeleton which makes more accurate structure to the actual RBC membrane. The cytoskeleton is modeled by triangular network of beads connected to each other by harmonic springs. The cytoskeleton is coupled with the lipid bilayer by using bolalipid like junction structures.

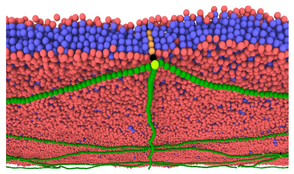

Figure 10. Head-tail model of RBC membrane

Pore formation was studied at various cytoskeleton concentrations and equibiaxial and non-equibiaxial loading conditions. The presence of cytoskeleton and transmembrane proteins confine pore formation and reduce total pore area. On the other hand, more complex, non-equibiaxial loading can result in higher number of pores in the membrane. These result will be used as the first step toward developing a multiscale predictive damage model.

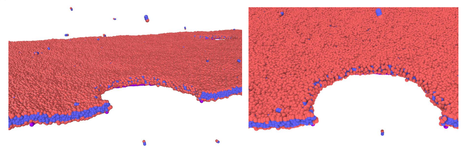

Figure 11. Orientation of the hydrophilic and hydrophobic poles of the head-tail model adjust themselves for a stable pore formation..

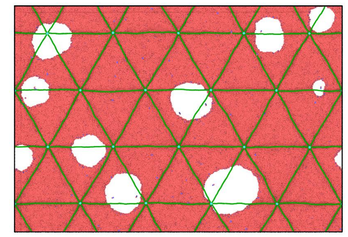

Figure 12. Pore formation in a patch of bilayer membrane due to equibiaxial loading (areal strain of 10.25%).

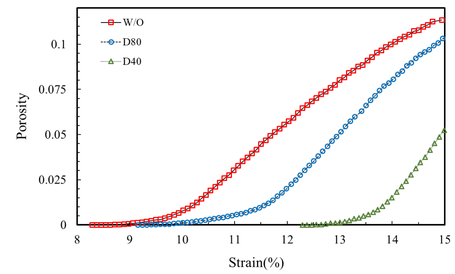

Figure 13. Cytoskeleton confines pore formation, Porosity (pore area over initial area) for lipid bilayer (W/O) and lipid bilayer with the cytoskeleton (D80 and D40: junction-to-junction distance of 80nm and 40nm)

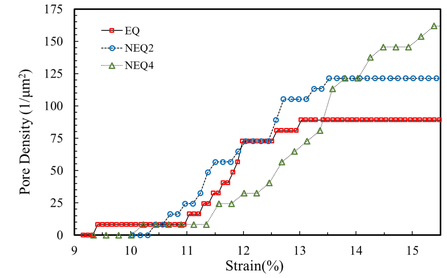

Figure 14. Complex loading condition increase pore density. Pore density for equibiaxial (EQ), and non-equibiaxial loading with two (NEQ2) and four (NEQ4) times more strain rate in x direction.

Conclusion

- One-particle-thick and head-tail models were used to study RBC large deformations and pore formation at different scales.
- Large deformation can result in cytoskeleton aggregation.
- Cytoskeleton confines the pore formation process. More cytoskeleton concentration results in less average pore radius and higher number of pores.
- Non-uniform loading leads to pore opening at higher strain level but results in larger pore area.

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