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Multiscale Modeling of Malaria

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Parasitic infectious diseases like malaria and certain hereditary hematologic disorders are often associated with major changes in the shape and viscoelastic properties of red blood cells. Such changes can disrupt blood flow and, possibly, brain perfusion, as in the case of cerebral malaria. In recent work on stochastic multiscale models—in conjunction with large-scale parallel computing—we were able to quantify, for the first time, the main biophysical characteristics of malaria, i.e., the dynamics and microrheology of malaria-infected blood flow in capillaries and small arteries. The same computational framework can be applied to other blood diseases, including sickle cell anemia and diabetes.

Background

A healthy human red blood cell is a discocyte, approximately 8 μm in diameter and 2 μm thick. The membrane of an RBC is made up of a phospholipid bilayer and a network of spectrin molecules (the cytoskeleton), which is connected to the bilayer by transmembrane proteins. The cytoskeleton is largely responsible for the shear elastic properties of the RBC and, with the spectrin filaments and the cytosol inside the membrane, determines the morphologic structure of the RBC. The parasite *Plasmodium falciparum*, the cause of one of the most serious forms of malaria, drastically affects the properties of the RBC membrane, resulting in a ten-fold increase in its shear modulus, and a spherical shape of the RBC at the later stages of intracell parasite development [1].

In addition, knobs that form on the surface of red blood cells invaded by *P. falciparum* (Pf-RBCs) serve as adhesion sites for binding both to other Pf-RBCs and to healthy RBCs. This enhanced cytoadherence of Pf-RBCs, in combination with their reduced deformability, can result in the obstructed blood flow characteristic of the hematologic disorders discussed here. Sickle cell anemia also causes dramatic changes in the shape and deformability of RBCs; combined with the increased internal viscosity, these changes affect the flow of sickled RBCs through capillaries, leading to flow occlusion [6].

The common problem in these hematologic disorders is the remodeling of the cytoskeleton and the corresponding changes in the structure and viscoelastic properties of individual RBCs. Studying their mechanical and rheological properties in vitro can thus contribute greatly to the understanding and possible discovery of new treatments for such diseases. To this end, new advanced experimental tools are valuable for determining the basic properties of single RBCs, both healthy and diseased, which we need in order to formulate multiscale methods for modeling blood flow in vitro and in vivo. For parasitic infectious diseases, powerful imaging techniques developed in recent years make it possible to observe details of parasite development inside an RBC and also to gain information about the properties of the cell components. Figure 1(a), an image obtained by a soft x-ray technique, shows the parasite *P. falciparum* inside an RBC during the ring stage of parasite development. The parasite and an elaborate structure extending from the parasite into the cell cytosol are clearly visible.

A number of numerical models have been developed recently to model the dynamics and rheology of red blood cells and of blood flow in small arteries, including a continuum description [10] and a discrete approximation both on the spectrin molecular level [2] and at the mesoscopic scale [9]. Some of the models, hindered by the assumption of a purely elastic membrane, are able to capture only the RBC mechanical response and cannot quantitatively represent realistic RBC rheology and dynamics. With fully continuum (fluid and solid) models, nontrivial coupling between nonlinear solid deformations and fluid flow can make the models computationally expensive. In addition, continuum models omit some mesoscopic- and microscopic-scale phenomena, such as membrane thermal fluctuations, that affect RBC rheology and dynamics. On the microscopic scale, detailed spectrin molecular models of RBCs are extremely limited by their high computational cost.

For these reasons, "semi-continuum" models of deformable particles that use immersed-boundary or front-tracking techniques are developing rapidly. In these models, a membrane is represented by a set of points that are tracked in Lagrangian fashion and coupled to an Eulerian discretization of the fluid domain. These models employ the same external (plasma) and internal (cytosol) fluids and do not take into account the differences in their viscosities.

Mesoscopic modeling of RBCs and blood flow seems to be the most effective approach for modeling malaria and other hematologic disorders. Such models can seamlessly represent the RBC membrane, cytoskeleton, cytosol, surrounding plasma, and even the parasite, as in the malaria-infected RBC shown in Figure 1.

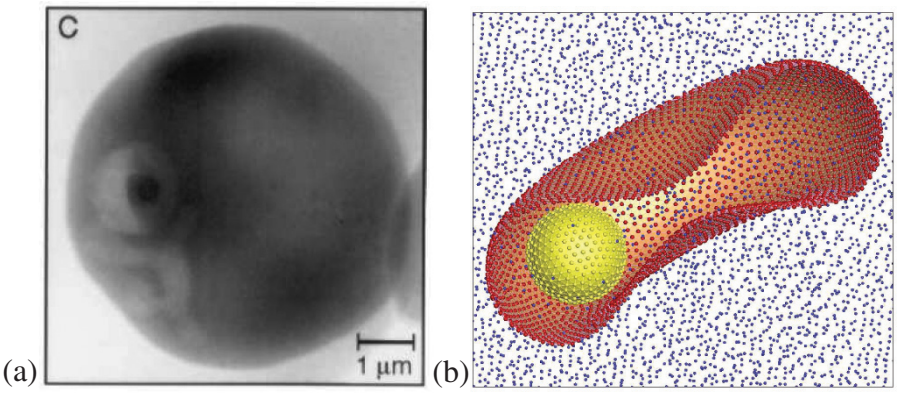

Figure 1. *(a) Soft x-ray micrograph of a* P. falciparum *malaria parasite, at the intra-erythrocytic ring stage, in a red blood cell. Reproduced from [8]. (b) The computational RBC model consists of particles connected by links. In the model the RBC is immersed in DPD (dissipative particle dynamics) fluid, with which it fully interacts through pairwise forces. The internal DPD fluid has a higher viscosity to match the viscosity of the RBC cytosol. The* P. falciparum *parasite is modeled as a rigid sphere, 2 μm in diameter. Courtesy of I.V. Pivkin.*

Multiscale Modeling via Dissipative Particle Dynamics

Dissipative particle dynamics (DPD) is a mesoscopic (Lagrangian) particle method in which each particle represents a *molecular cluster* rather than an individual atom and can be thought of as a soft "lump" of fluid. It was first proposed as a momentum-conserving alternative to Brownian dynamics for modeling polymers [5]. A first-principles derivation of the DPD method from the Liouville equation

is presented in [7], following the Mori–Zwanzig formulation to introduce the projector operator method, which provides the theoretical basis for a systematic coarse-graining procedure.

The DPD system consists of point particles characterized by mass, position, and velocity. Typically, each DPD particle represents ten molecules, with the number depending on the aggressiveness of the coarse-graining strategy for a specific problem. DPD particles interact in a pairwise fashion through three forces: conservative, dissipative, and random. The conservative force is similar to the force used in molecular dynamics, but effective soft potentials employed in the DPD method reflect averages over the microstructure of each particle. The dissipative and random forces in a pair are connected through the dissipation–fluctuation theorem and form a *local* thermostat, unlike in molecular dynamics, where global thermostats are typically used. The time evolution of the velocities and particle positions is determined by Newton's second law of motion, which leads to a system of stochastic nonlinear ordinary differential equations that differ from the deterministic ODEs in molecular dynamics. These stochastic equations of motion can be integrated with a modified velocity-Verlet algorithm; for systems governed by mixed hard–soft potentials (as in modeling DNA or proteins in a solvent), subcycling techniques can also be employed for computational efficiency.

Multiscale Red Blood Cell (MS-RBC) Model. The average equilibrium RBC is biconcave, and the shape can be represented analytically. In simulations, we generate the membrane network structure by triangulating the unstressed equilibrium shape, in essence mimicking the triangulated structure of the cytoskeleton. The cell shape is first imported into a grid generator to produce an initial triangulation based on the advancing-front method. In subsequent free-energy relaxation, the diagonals of the quadrilateral elements formed by two adjacent triangles are flipped, while the vertices are constrained to move on the prescribed surface. The relaxation procedure includes only the elastic in-plane and bending energy components described below.

Figure 2 shows the membrane model represented by a set of points $\{\mathbf{x}\}_i$, $i \in 1 \ldots N_v$, that are the vertices of a two-dimensional triangulated network on the RBC surface. About 30,000 vertices are required for the triangulated surface to match the molecular dimensions of the spectrin filaments of the cytoskeleton (with about 70 nanometers between vertices). Given that one cubic millimeter of blood contains about five million RBCs, tremendous computational resources will be required to simulate even tiny capillaries. Hence, in practical simulations we work with coarse-grained models, as in Figure 2, using consistently effective parameters that depend on the degree of coarse-graining, as explained in [9].

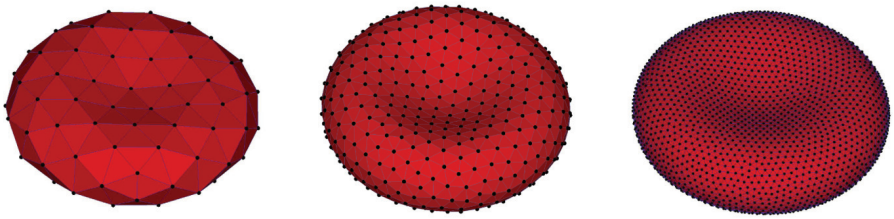

Figure 2. *Multiscale RBC membrane model with, from left to right, N_v = 100, 500, and 3000.*

The vertices are connected by N_s edges, which form N_t triangles. Based on this geometry, we define the potential energy of the system in terms of the in-plane and bending energies, along with energies from area and volume conservation. The in-plane elastic energy mimics the elastic spectrin network and is expressed in terms of viscoelastic spring parameters; a wormlike chain or a FENE polymer model can be used. The bending energy represents the resistance of the lipid bilayer to deformation. The area and volume conservation constraints, which account for the area-incompressibility of the lipid bilayer and the incompressibility of the inner cytosol, respectively, are added to the total energy. Linear analysis of the regular hexagonal network that has these energies yields a relation between macroscopic elastic properties (shear, area-compression, and Young's modulus) of the network and microscopic model parameters.

The model just described defines a purely elastic membrane; the RBC membrane, however, is known to be viscoelastic. To incorporate viscosity in the model, the spring definition is modified by adding viscous contribution through dissipative and random forces. Such a formulation fits naturally into the DPD method, of which interparticle dissipative interactions are an intrinsic part. A detailed description and discussion of the RBC model can be found in [3].

Application to Malaria Modeling

Plasmodium falciparum causes several million deaths per year. Pf-RBCs experience progressive changes in mechanical and rheological properties as well as in morphology during intraerythrocytic parasite development, which proceeds in three stages: ring → trophozoite → schizont (from earliest to latest).

Progression through these stages involves considerable stiffening of Pf-RBCs, as found in stretching experiments with optical tweezers and in monitoring of membrane fluctuations in diffraction phase microscopy [1]. Development of the parasite also results in the formation of vacuoles inside RBCs and, possibly, changes in the cell volume. Thus, Pf-RBCs, after maintaining their biconcavity in the two earlier stages, often have a "near spherical" shape in the final (schizont) stage. These changes greatly affect the rheological properties and dynamics of Pf-RBCs, and can lead to obstruction of small capillaries, impairing the ability of RBCs to circulate.

Figure 3 shows simulation results for Pf-RBCs at different stages of parasite development. For the results shown, the multiscale RBC model was used with 500 points. The curve for the "near-spherical" schizont stage corresponds to stretching an ellipsoidal shape, and to a membrane shear modulus of 40 μN/m

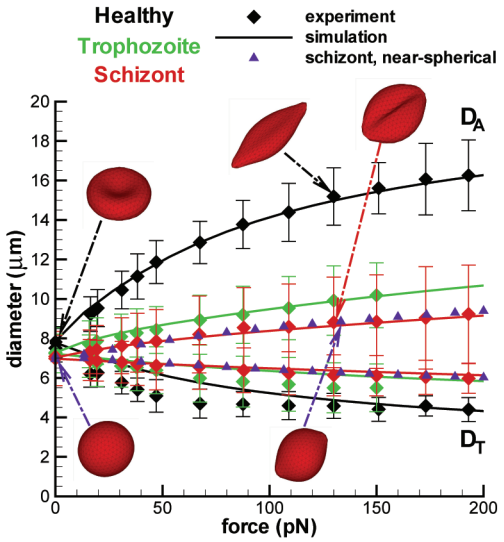

Figure 3. *Stretching response of Pf-RBCs with the multiscale RBC model for different stages compared with experiments. D_A and D_T refer to the axial and transverse diameters. From [4].*

to match the stress–strain response with the experiment—i.e., smaller than 60 μN/m for the biconcave-shape simulation.

We also performed simulations of blood flow in malaria modeled as a suspension of healthy and Pf-RBCs at the trophozoite stage, with a hematocrit $H_t = 0.45$. (Hematocrit is the percentage of red blood cells (by volume) in the blood after all other cells/proteins are removed.) Several parasitemia levels (percentage of Pf-RBCs with respect to the total number of cells in a unit volume), from 5% to 100%, are considered for vessels from 10 to 20 μm in diameter. The inset in Figure 4 is a snapshot of RBCs flowing in a tube 20 μm in diameter at a parasitemia level of 25%.

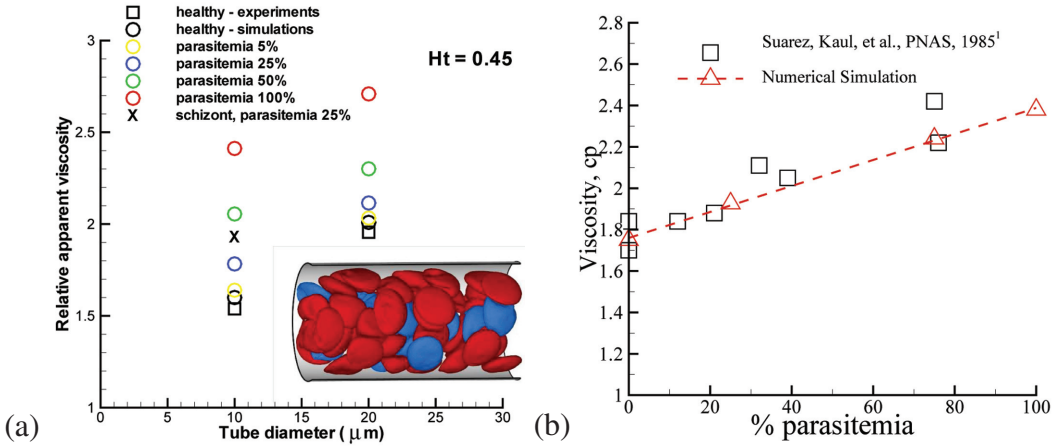

Figure 4. *Flow resistance in malaria. (a) Healthy RBCs (red) and Pf-RBCs (blue) in Poiseuille flow in a tube of diameter D = 20 μm; $H_t = 0.45$, and the parasitemia level is 25%. The relative apparent viscosity of blood in malaria is plotted for various parasitemia levels and tube diameters. The symbol "x" corresponds to the schizont stage, with a near-spherical shape. Experimental data is from the empirical fit by Pries et al. [11]. Courtesy of D. Fedosov. (b) Bulk viscosity versus parasitemia level for 30% hematocrit using a Couette device setup at a shear rate of 230 s^{-1}. Measurements from [12] are indicated by square symbols, the simulations by triangles. Courtesy of H. Lei.*

The main result in Figure 4(a) is given by the plot of the relative apparent viscosity in malaria—a measure of flow resistance—at different parasitemia levels. The effect of parasitemia level appears to be more prominent for small diameters and high hematocrit values. Thus, at $H_t = 0.45$, blood flow resistance in malaria may increase by as much as 50% in vessels about 10 μm in diameter and by as much as 43% in vessels 20 μm in diameter. These increases do not include any contributions from the interaction of Pf-RBCs with the glycocalyx; such important interactions are complex, as they may include cytoadhesion (see [4]).

Figure 4(b) also presents the bulk viscosity of infected blood (schizont stage), simulated in Couette flow—in a rheometer-type device—at shear rate $\gamma = 230\ s^{-1}$. The DPD simulations compare favorably with experimental data obtained with a corresponding rheometer in [12]. These validated predictions were obtained without an explicit adhesion model between Pf-RBCs. Such cell–cell interactions do not seem to be important at this high shear rate.

Outlook

Our work demonstrates that, starting from single-RBC measurements—made possible by modern experimental techniques, such as optical tweezers and microfluidics—accurate predictions of the collective dynamics and microrheology of healthy and infected blood can be made without further "tuning" of the model parameters. In the work described here, we treat whole blood as a suspension of RBCs in plasma, hence ignoring the effects of white cells (about 0.7%) and platelets (less than 0.5%), as well as the effects of other proteins in the plasma; we can model fibrinogen implicitly, using effective aggregation potential that leads to rouleaux formation. From the numerical modeling standpoint, there is no particular difficulty in also modeling these other cells, which are significant in specific biomedical studies, e.g., in thrombosis or an immune response. From the biophysical viewpoint, however, their presence is not important for the whole-blood rheological properties.

In ongoing work we have also quantified the biophysical characteristics of sickle cell anemia, in which shape changes are very important and may occur dynamically through continuous hemoglobin polymerization in deoxygenated states. Spherocytosis and elliptocytosis are hereditary diseases with similar effects. In the former, RBCs become spherical and smaller in diameter, and carry much more hemoglobin than healthy RBCs. In the latter, RBCs are elliptical or oval and of reduced deformability. A similar computational framework can be used to study these hematologic disorders, as well as other blood pathologies in patients with diabetes or AIDS.

Acknowledgments

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