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Quantifying the biophysical characteristics of *Plasmodium-falciparum*-parasitized red blood cells in microcirculation

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The pathogenicity of *Plasmodium falciparum* (Pf) malaria results from the stiffening of red blood cells (RBCs) and its ability to adhere to endothelial cells (cytoadherence). The dynamics of Pf-parasitized RBCs is studied by three-dimensional mesoscopic simulations of flow in cylindrical capillaries in order to predict the flow resistance enhancement at different parasitemia levels. In addition, the adhesive dynamics of Pf-RBCs is explored for various parameters revealing several types of cell dynamics such as firm adhesion, very slow slipping along the wall, and intermittent flipping. The parasite inside the RBC is modeled explicitly in order to capture phenomena such as “hindered tumbling” motion of the RBC and the sudden transition from firm RBC cytoadherence to flipping on the endothelial surface. These predictions are in quantitative agreement with recent experimental observations, and thus the three-dimensional modeling method presented here provides new capabilities for guiding and interpreting future in vitro and in vivo studies of malaria.

adhesion | erythrocyte | malaria | mechanical properties | dissipative particle dynamics

Red blood cells parasitized by *Plasmodium falciparum* (Pf-RBCs) undergo irreversible changes in structure and biophysical characteristics, which can lead to drastically altered blood circulation. The membrane shear modulus of infected RBCs may increase up to ten-fold causing capillary occlusions (1, 2), thereby resulting in substantial increase in resistance to blood flow. Such effects may be intensified due to the enhanced cytoadherence of Pf-RBCs to the vascular endothelium (3–6). This adherence of Pf-RBCs is believed to be the main cause of bleeding complications in cerebral malaria due to blockages of small vessels in the brain (7). Unlike the extensive research on leukocytes, very few in vitro experiments (8–11) have examined the adhesive dynamics of Pf-RBCs. For example, in ref. 8 the walls of microfluidic channels were coated with purified protein ligands participating in cytoadherence (e.g., ICAM-1) and with mammalian Chinese hamster ovary (CHO) cells expressing such ligands. Purified ICAM-1 caused rolling or flipping of Pf-RBCs without detachment or arrest of RBC motion. In contrast, mammalian CHO cells seem to result mostly in a firm attachment of Pf-RBCs with sporadic complete detachment. This difference in behavior and the complicated adhesive dynamics of Pf-RBCs have not been studied quantitatively. More broadly, there have not been any quantitative studies of the dynamics of RBCs in malaria to investigate the rheology and flow resistance in addition to the reported new adhesive dynamics.

Recent progress in multiscale numerical modeling (12–14) allows us to model soft matter, and RBCs in particular, at sufficient detail, i.e., simulating nanometer scales while simultaneously capturing the large scale dynamics. We have developed and validated a Dissipative Particle Dynamics (DPD) model (12, 14–16) that can accurately simulate the properties and dynamic behavior of healthy RBCs as well as Pf-RBCs. The multiscale model can represent a RBC at the spectrin level with 30,000 points (17)

or at a coarser level with 500 points by proper scaling of the physiologically correct parameters (12, 14, 15); hence, no ad hoc calibration is required. The predictive capability of the DPD model has been demonstrated in comparisons with microfluidic experiments that probe controlled pressure-velocity relationships of (healthy) RBC flow through microchannels whose inner openings mimic the smallest dimensions for RBC passage in the microvasculature (16). In addition, we have extended the adhesive dynamics model of (18, 19) to the DPD framework, and validated it by simulating the adhesive dynamics of leukocytes for which extensive experimental results exist (20, 21). In summary, in the current work using DPD we model the RBC membrane as a viscoelastic material, the solid Pf-parasite, the fluid inside the cells and the exterior plasma, as well as the functionalized microchannel walls. The model parameters include the membrane shear modulus μ_0 , the membrane bending rigidity k_c , the membrane viscosity η_m , and the interior/exterior η_i/η_o fluid viscosities; see *Methods* and *SI Text* for more details.

Results

We first validate our RBC model in health and disease with physiologically correct values of all parameters using data from optical tweezers experiments. Subsequently, using the same set of parameters we investigate the dynamics of Pf-RBCs at different parasitemia levels and quantify the different modes of adhesive dynamics in the presence of ICAM-1 coated wall surfaces.

Increased Stiffness of Pf-Parasitized RBCs. In malaria disease, progression through the parasite development stages (ring \rightarrow trophozoite \rightarrow schizont) leads to a considerable stiffening of Pf-RBCs compared to healthy ones (22, 23). Furthermore, in the schizont stage the RBC shape becomes near-spherical whereas in the preceding stages RBCs maintain their biconcavity. Fig. 1 shows simulation results for healthy RBCs and Pf-RBCs at different stages of parasite development compared with optical tweezers experiments (23). The simulation results were obtained with a stress-free multiscale RBC model (see *Methods*) with 500 points, shear modulus $\mu_0 = 6.3 \mu\text{N/m}$ for the healthy RBC, 14.5 for the ring stage, 29 for the trophozoite, and 60 $\mu\text{N/m}$ for the schizont; these values are consistent with the experiments of refs. 22, 23. The bending rigidity for all cases is set to $2.4 \times 10^{-19} \text{ J}$, which is the value of bending rigidity for healthy RBCs, as the membrane bending stiffness for different stages is not known. The curve for the schizont stage marked as “near-spherical” corresponds to stretching an ellipsoidal shape with

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$H_t = 0.45$. 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 in vessels with diameters 10 and 20 μm . The inset of Fig. 3 shows a snapshot of RBCs flowing in a tube of diameter 20 μm at a parasitemia level of 25%, see also [Movie S1](#). The main result in Fig. 3 is given by the plot of the relative apparent viscosity in malaria—a measure of flow resistance—obtained at different parasitemia levels. The effect of parasitemia level appears to be more prominent for small diameters and high H_t values. Thus, at $H_t = 0.45$ blood flow resistance in malaria may increase up to 50% in vessels of diameters around 10 μm and up to 43% for vessel diameters around 20 μm . These increases do not include any contributions from the interaction of *Pf*-RBCs with the glycocalyx (27, 28); such important interactions are complex as they may include cytoadhesion which we model next.

Adhesive Dynamics. The adhesive dynamics of *Pf*-RBCs in shear flow was studied for different values of wall shear stress (WSS) and compared with the experiments of ref. 8 for the wall coated with purified ICAM-1. Fig. 4 (upper left) shows several successive snapshots of a simulated cell rolling along the wall. Small blue particles are added as tracers for visual clarity, and distinct RBC snapshots are separated by shifting their x coordinate. The dynamics of the *Pf*-RBCs is characterized by a flipping behavior initiated at first by the cell peeling off the wall due to the hydrodynamic force after flat RBC adhesion (the first snapshot in the plot; see also [Movie S2](#)). After most of the initial cell wall contact area is peeled off, the RBC flips over onto its other side facilitated by the remaining small wall contact area. During these steps, *Pf*-RBCs undergo large membrane deformations as illustrated in the plot and in [Movie S2](#). Similar flipping behavior and large membrane deformations (including membrane buckling) were also found in the experiments (8).

WSS appears to be the key parameter governing the *Pf*-RBC adhesive dynamics, because adhered RBCs are driven by fluid stresses and roll along the wall with a much smaller velocity than the flow velocity. Several initial simulations with varying WSS and other parameters fixed revealed that *Pf*-RBCs may exhibit firm adhesion at a WSS lower than 0.317 Pa while they can completely detach from the wall at higher values. Systematic visualizations

showed that *Pf*-RBC detachment at high WSS occurs during the relatively fast motion of RBC flipping, because the contact area is then minimal. However, in the experiments of ref. 8 *Pf*-RBCs moved on a surface coated with purified ICAM-1 showing persistent and stable rolling over long observation times for a wide range of WSS between 0.2 Pa and 2 Pa. This observation suggests a stabilizing mechanism for the rolling of *Pf*-RBCs at high shear stresses. Such behavior is not surprising because it is known that leukocyte adhesion can be actively regulated with flow conditions and with biochemical stimuli (29, 30).

To stabilize RBC binding at high shear stresses we need to improve the model by allowing the bond spring constant (k_s) to vary with WSS; here, for simplicity we assume linear dependence of k_s on WSS. Fig. 4 (lower left) presents the average rolling velocity of *Pf*-RBCs compared with experiments of cell rolling on a surface coated with purified ICAM-1 (8). The simulated average velocities show a near-linear dependence on the shear stress, and are in good agreement with the experiments. The discrepancy at the highest simulated shear stress suggests a further strengthening of cell-wall bond interactions. However, the simulated values remain between the 10th and the 90th percentiles found in experiments.

In general, the adhesive behavior of *Pf*-RBCs, explored by means of numerical simulation for various parameters, revealed several types of cell dynamics such as firm adhesion, RBC peeling off the surface followed by flipping from one side to the other or by detachment from the wall, and very slow slipping along the wall. However, close examination of the video containing an example of RBC adhesive dynamics on the mammalian CHO cells from experiments (8) shows firm adhesion of *Pf*-RBCs for some time followed by *sudden* detachment. In contrast, firm adhesion in simulations appears always to be stable with no detachment within the simulation time of approximately 30 s. In experiments the *Pf*-RBC motion before the detachment displays very slow slipping along the surface due to the flow and random collisions with other flowing RBCs. Hence, it is likely that the sudden complete detachment from the wall is caused by the RBC slipping into a wall region with a limited number of ligands available for binding due to imperfect coating. To verify this hypothesis we ran a simulation in which the ligand sites were removed from the wall area between 30 μm and 40 μm in the flow direction. Fig. 4 (lower right) presents the *Pf*-RBC instantaneous velocity (green curve) corresponding to slow slipping along the surface continued up to an x coordinate (stream-wise position) between 30 μm and 40 μm , where a complete cell detachment occurs due to absence of ligands for binding, in agreement with the *Pf*-RBC dynamics on the mammalian CHO cells found in experiments (8). No other change in physical parameters of cell adhesion have been found to reproduce these dynamics.

Next, we model explicitly the effect of the solid parasite inside the *Pf*-RBCs. Recent experiments (22) suggest that the volume of cytosol may be reduced threefold in the later stages of intracellular parasite development compared to healthy RBCs, indicating that the parasite takes up considerable volume within a *Pf*-RBC. Hence, a sufficiently large parasite provides a rigid backbone within a *Pf*-RBC to strongly affect its adhesive dynamics. In experiments in ref. 9 it was observed that the parasite is attached to one side, especially in the ring and trophozoite stages, causing a structural anisotropy in the *Pf*-RBC that affects cytoadherence. The parasite is modeled as a collection of DPD particles uniformly distributed within a cylindrical volume of radius 3.3 μm and height 0.2 μm . These particles are placed inside the modeled RBC and constrained to undergo rigid motion. To prevent the parasite body from crossing the RBC membrane, we introduce Lennard-Jones interactions between the parasite body particles and membrane vertices; however, the parasite swims freely in the RBC cytosol. The number of DPD particles to represent the RBC cytosol is reduced according to the volume occupied by the

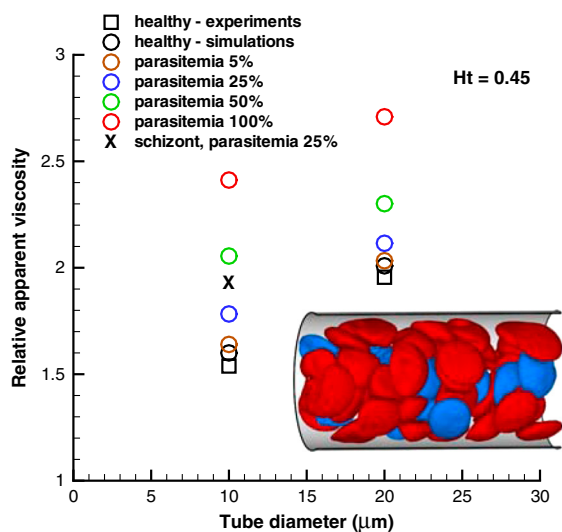


Fig. 3. Flow resistance in malaria: Healthy (red) and *Pf*-RBCs (blue) in Poiseuille flow in a tube of diameter $D = 20 \mu\text{m}$. $H_t = 0.45$, parasitemia level 25%. Plotted is the relative apparent viscosity of blood in malaria for various parasitemia levels and tube diameters. Symbol “x” corresponds to the schizont stage with a near-spherical shape. Experimental data from the empirical fit by Pries et al. (26).

membrane and ligands on the wall. In the simulations, distributions of both receptors and ligands are fixed, and are nearly homogeneous with approximately the same area occupied by each receptor or each ligand. A scatter in behavior among distinct RBCs in the simulations is solely related to the stochastic nature of the adhesive model. However, in experiments irregular distributions of receptors and ligands are likely to significantly contribute to scatter in RBC adhesive dynamics.

The parasite body constrains the RBC membrane by supplying a rigid support, which forces RBC flipping without substantial bending. The presence of a rigid body inside a RBC significantly affects the RBC adhesive dynamics resulting in behavior more erratic compared with the more regular adhesive dynamics of RBCs with no parasites. A thin disk to represent the parasite body was considered; however, other geometrical forms or sizes of the parasite may have a different effect on RBC adhesive dynamics. Therefore, an experimental characterization of the parasite geometry for different stages of parasite development would be of great interest. In addition, the modeled parasite body was freely suspended in the RBC cytosol, while under real conditions it is likely that the parasite is attached to the membrane, because it exposes adhesive proteins on the membrane surface to mediate binding to the wall. These unresolved issues require further experimental and numerical investigation.

Methods

Simulation Method. The DPD method (33) is a particle-based mesoscopic simulation technique, where a simulated system consists of N point particles. Each particle corresponds to a collection of atoms or molecules rather than an individual atom. DPD particles interact through pair wise soft potentials and move according to the Newton's second law of motion; see also *SI Text*.

Membrane Model. The RBC membrane is modeled by 500 discrete points, which are the vertices of a triangular network of springs on the membrane surface. Such network models have been used extensively to simulate mem-

branes, e.g., see ref. 34. The network of fixed connectivity provides the elastic and the viscous response of a RBC because a "dashpot" is attached to each spring. The RBC model also includes bending energy between neighboring triangular plaquettes and area and volume constraints. We use a "stress-free" model (14, 15) which eliminates artifacts of irregular triangulation. The "stress-free" model is obtained by simulation annealing such that each spring assumes its own equilibrium spring length adjusted to be the edge length after triangulation. RBC-fluid boundary conditions are enforced through bounce-back reflections of fluid particles on the membrane triangles and by a proper setting of interactions between fluid particles and RBC vertices. More details on the RBC model can be found in the *SI Text*.

Adhesive Dynamics. Adhesive dynamics is simulated with the stochastic bond formation/dissociation model similar to ref. 18. The bonds are modeled as linear springs and their formation k_{on} and dissociation k_{off} rates depend on the separation distance between the RBC receptors and ligands distributed on the wall as a square lattice with the lattice constant of 0.5 μm . The receptor and ligand densities in simulations may be different from those in experiments (8). However, we note that receptor-ligand interactions in simulations correspond to effective adhesive interactions of Pf-RBCs with the wall. The simulated receptor-ligand interactions do not correspond to actual molecular bonds and may represent a number of existing molecular bonds. Adhesive dynamics in simulations proceeds by: (i) checking for potential dissociation of existing bonds with probability $1 - \exp(-k_{off}\Delta t)$, where Δt is the time step, (ii) testing unbound ligands for potential bond formation with probability $1 - \exp(-k_{on}\Delta t)$, and (iii) applying forces of all existing bonds. More details on the adhesive dynamics and model parameters can be found in *SI Text*.

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