

# Predicting human blood viscosity in silico

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The viscosity of blood has long been used as an indicator in the understanding and treatment of disease, and the advent of modern viscometers allows its measurement with ever-improving clinical convenience. However, these advances have not been matched by theoretical developments that can yield a quantitative understanding of blood's microrheology and its possible connection to relevant biomolecules (e.g., fibrinogen). Using coarse-grained molecular dynamics and two different red blood cell models, we accurately predict the dependence of blood viscosity on shear rate and hematocrit. We explicitly represent cell-cell interactions and identify the types and sizes of reversible rouleaux structures that yield a tremendous increase of blood viscosity at low shear rates. We also present the first quantitative estimates of the magnitude of adhesive forces between red cells. In addition, our simulations support the hypothesis, previously deduced from experiments, of yield stress as an indicator of cell aggregation. This non-Newtonian behavior is analyzed and related to the suspension's microstructure, deformation, and dynamics of single red blood cells. The most complex cell dynamics occurs in the intermediate shear rate regime, where individual cells experience severe deformation and transient folded conformations. The generality of these cell models together with single-cell measurements points to the future prediction of blood-viscosity anomalies and the corresponding microstructures associated with various diseases (e.g., malaria, AIDS, and diabetes mellitus). The models can easily be adapted to tune the properties of a much wider class of complex fluids including capsule and vesicle suspensions.

blood rheology | blood modeling | shear thinning | aggregation force | dissipative particle dynamics

Rheological and material properties of cell, capsule, and vesicle suspensions have many applications in medicine, biology, engineering, and materials science. One of the main examples of such suspensions is blood, which consists of RBCs, predominant by volume, and a small fraction of other cells and proteins suspended in the plasma. Understanding blood flow and its relation to cellular properties and interactions may lead to advances in biomedical applications (e.g., drug delivery, blood substitutes). Moreover, a change in blood rheological and flow properties is often associated with hematological diseases or disorders (e.g., sickle-cell anemia, malaria), and therefore the viscosity of blood has long been used as an indicator in the understanding and treatment of disease.

Modern rheometry techniques and instruments yield reliable measurements of macroscopic properties of cell suspensions with ever-improving convenience—for example, the bulk properties of blood measured in various laboratories (1–6). Virtually all blood-viscosity measurements are necessarily *in vitro*, and before newly drawn blood is introduced into a viscometer it must at least be stabilized with an anticoagulant, which is then called “whole blood.” Under flow conditions at small deformation rates, the RBCs in whole blood have been observed to aggregate into structures called “rouleaux,” which resemble stacks of coins (1, 7–9). The aggregation process appears to be strongly correlated to the presence of the plasma proteins (7, 9). Experiments with washed RBCs resuspended in pure saline, to which fibrinogen was added progressively (7), showed a tremendous viscosity increase at low

deformation rates with respect to fibrinogen concentration. In addition, such suspensions exhibit a yield stress (1, 10, 11)—i.e., a threshold stress for flow to begin.

However, these advances have not been accompanied by theoretical developments that can yield quantitative predictions of rheological and flow properties of blood. Recent theoretical and numerical studies focused mostly on the behavior of a single RBC in various flows (12–16). Several studies have been performed to simulate a suspension of multiple cells (16–19) in tube flow. So far, the connection between the rheology of a cell suspension and its microscopic properties on a single-cell level, such as structure or arrangement, cell viscoelastic properties, and local dynamics, is not well understood. In addition, cell suspensions are often further complicated by intrinsic cell interactions (e.g., RBC aggregation; refs. 1, 7–9). In this paper, we will establish such a link between bulk properties and microstructure, and will focus on the *quantitative* prediction of rheological properties and dynamics of blood flow by employing multiscale modeling of interacting red blood cells.

## Results and Discussion

We consider suspensions of RBCs to mimic the experimental set up of washed RBCs suspended in pure saline, to which fibrinogen was added progressively (7), and we will refer to them as erythrocyte suspensions (ES). We simulate them with dissipative particle dynamics (DPD), a coarse-grained version of molecular dynamics suited to the seamless modeling of liquids and soft matter (14, 20, 21). Two different cell models are employed. The first, a multiscale RBC model (MS-RBC) (15) represents the RBC membrane with a few hundred DPD particles connected by viscoelastic springs into a triangular network in combination with out-of-plane elastic bending resistance, similar to the mesoscopic model in Refs. 12, 18, and 22. The characteristic biconcave RBC shape is achieved by imposition of constraints for constant membrane area and constant cell volume. Fitting of the model parameters is performed through a number of static and dynamic experiments on single real RBCs (15) and no further adjustment is made for the RBCs in suspension. Because simulations with MS-RBC are computationally expensive, we also employ a low-dimensional model (LD-RBC) of an RBC (23) for efficiency in parametric studies. LD-RBC is constructed as a closed torus-like ring of only 10 large hard colloidal particles, see *Methods* for more details. LD-RBC allows exploration of simulated blood flows over a wide range of hematocrits at computational costs considerably below those for their multiscale counterparts. In addition to the LD-RBC and MS-RBC models, we developed an aggregation model to reproduce the reversible rouleaux formation and destruction, which is essential to capture blood flow behavior, especially at low shear rates. Next, we present results for the ES viscosity with

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and without aggregation, rouleaux formation and magnitude of aggregation forces, yield stress, and the micro-to-macro link in ES.

**In Silico Versus in Vitro Blood Viscosity.** The experimental bulk viscosities of well-prepared nonaggregating ES and of whole blood were measured for various hematocrit values ( $H$ ) at physiological temperature  $37^\circ\text{C}$  in refs. 1–3. The blood viscosity in our work was derived from simulations of plane Couette flow using the Lees–Edwards periodic boundary conditions for both the MS-RBC and the LD-RBC suspensions. The shear rate and the cell density in our simulations were verified to be spatially uniform on average over time, and the viscosities were computed, with and without aggregation, as functions of the shear rate over the range  $0.005\text{--}1,000.0\text{ s}^{-1}$  (this corresponds to the range of dimensionless shear rate or capillary number  $\eta\dot{\gamma}D/Y$  between  $2.5 \times 10^{-6}$  and  $0.5$ , where  $\eta$  is the solvent viscosity,  $D$  is the RBC diameter, and  $Y$  is the membrane Young’s modulus). Fig. 1A shows the relative viscosity (RBC suspension viscosity normalized by the viscosity of the suspending media) against shear rate at hematocrit  $H = 45\%$ . The MS-RBC model predictions are in excellent agreement with the blood viscosities measured in three different laboratories (1–3). The ES model, consisting only of RBCs in suspension, clearly captures the effect of aggregation on the viscosity at low shear rates and suggests that cells and molecules other than RBCs have little effect on the viscosity, at least under healthy conditions. The LD-RBC model underestimates somewhat the experimental data, but is generally in good agreement over the whole range of shear rates, and again demonstrates the effect of aggregation. The agreement is remarkable in view of the simplicity and economy of that model. Errors in simulated viscosities shown in Fig. 1A are approximately 30% for the shear rate  $\dot{\gamma} = 0.014\text{ s}^{-1}$  and decrease rapidly with the increase of  $\dot{\gamma}$ , becoming about 1–3% at high shear rates.

The dependence of whole blood and ES viscosity on hematocrit is demonstrated in Fig. 1B. The curves are measured viscosities as a function of  $H$  at constant shear rate by Chien et al. (2), and the points are calculated with the LD-RBC model. The plot clearly shows how the latter captures the (hematocrit)  $H$  dependence on viscosity, and that the model again demonstrates aggregation to be crucial for a quantitative account of the difference between the viscosity of whole blood and that of washed ES.

Recent attempts in modeling (24, 25) of two-cell and multiple-cell aggregates (17) simulated only their flow behavior. Specifically, in ref. 17, the link of viscosity to RBC aggregation was investigated, but the viscosity predictions failed to capture the steep rise of that function at low shear rates.

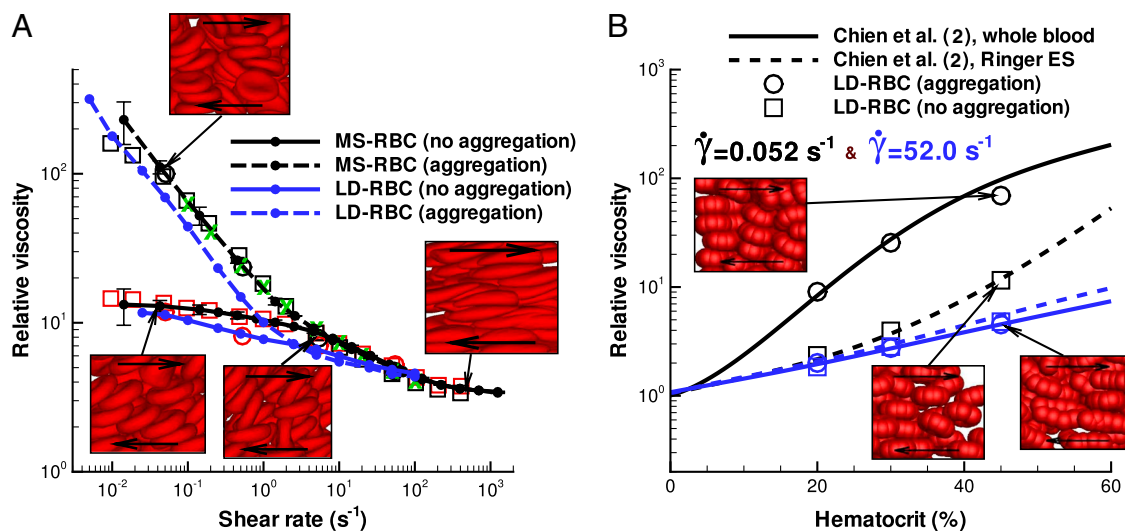
**Reversible Rouleaux Formation.** The formation of rouleaux in blood occurs in equilibrium and at sufficiently small shear rates, whereas large shear rates result in immediate dispersion of fragile RBC structures. Experimentally, aggregation is observed (1, 4, 26) to be a two-step process: the formation of a few RBCs into short linear stacks, followed by their coalescence into long linear and branched rouleaux. As the shear rate increases, the large rouleaux break up into smaller ones, and at higher values, the suspension ultimately becomes one of monodispersed RBCs (27). This process then reverses as the shear rate is decreased.

This typical formation–destruction behavior of rouleaux is consistent with the results of our simulations using both the LD-RBC and the MS-RBC models as shown in Fig. 2 (see *SI Text*). At low shear rates (left frames), the initially dispersed RBCs aggregate into large rouleaux of up to about 20 RBCs; as the shear rate is increased to moderate values (middle frames), these structures are reduced in size until at high rates (right frames) they are dispersed almost completely into individual RBCs. Reversibility is demonstrated by reduction of the shear rate to the formation value, at which point individual RBCs begin to reaggregate.

**Yield Stress and Aggregation.** Whole blood is believed to exhibit a yield stress (i.e., a threshold stress for flow to begin) (1, 10, 11), but this has been difficult to confirm experimentally or theoretically. The most reproducible yield stresses for whole blood are those extrapolated to zero shear rate from viscometric data on the basis of Casson’s equation given by (28)

$$\tau_{xy}^{1/2} = \tau_y^{1/2} + \eta^{1/2}\dot{\gamma}^{1/2}, \quad [1]$$

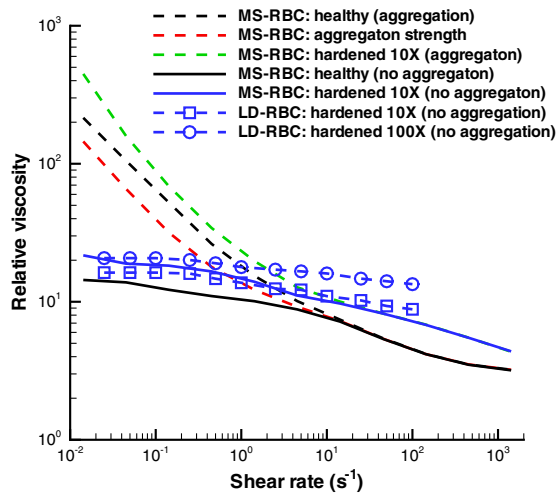
where  $\tau_y$  is a yield stress and  $\eta$  is the suspension viscosity at large  $\dot{\gamma}$ . Note that when the yield stress  $\tau_y$  vanishes, Eq. 1 reduces to the Newtonian liquid. The assumptions of Casson’s relation appear to hold at least at low shear rates, which was successfully demonstrated for pigment-oil suspensions (28), Chinese ovary hamster



**Fig. 1.** Validation of simulation results for whole blood and Ringer ES. (A) Plot of non-Newtonian relative viscosity (the cell suspension viscosity normalized by the solvent viscosity) as a function of shear rate at  $H = 45\%$  and  $37^\circ\text{C}$ . Simulated curves of this work, as indicated and experimental points as follows: Whole blood: green crosses, Merrill et al. (1); black circles, Chien et al. (2); black squares, Skalak et al. (3). Ringer ES: red circles, Chien et al. (2); red squares, Skalak et al. (3). Error bars on the MS-RBC viscosity curves reflect one standard deviation and each point on the simulated curves corresponds to a single simulation. (B) Plot of relative viscosity as a function of hematocrit ( $H$ ) at shear rates  $0.052$  (black) and  $52.0$  ( $\text{s}^{-1}$ ): simulated (LD-RBC points), and Chien et al. (2) experimental fits for whole blood (solid lines), and Ringer ES (dashed lines).







**Fig. 5.** Tunable properties of cell suspensions. MS-RBC and LD-RBC models with  $H = 45\%$ . Black lines are viscosities of healthy blood with and without aggregation by MS-RBC. Red line illustrates a decrease in cell aggregation strength reduced twice. Blue and green lines represent the viscosities of hardened RBC suspensions with  $10\times$  (MS-RBC and LD-RBC) and  $100\times$  (LD-RBC) higher Young's modulus than that in health. Each point on the simulated curves corresponds to a single simulation.

the solvent viscosity, material properties of suspended cells, and intercell aggregation interactions. Fig. 5 shows several examples of the tunable properties of cell suspensions. Results for cells with 10 and 100 times higher Young's modulus than healthy RBCs (blue and green curves) show a considerable increase of viscosity. Hardened RBCs are known (33, 34) to increase their suspension viscosities, and they are highly relevant in many hematologic disorders and diseases, e.g., malaria, sickle-cell anemia, spherocytosis. In addition, aggregating suspensions of stiffer cells show a steeper rise in viscosity at low shear rates resulting in a substantially higher yield stress. Fig. 5 also illustrates the expected decrease, relative to whole blood, in the suspension viscosity at low shear rates (red curve) due to a twofold reduction in the aggregation strength ( $D_e$ , see *Methods*). The significant change in viscosity observed above implies a strong dependence of flow properties on cell deformability and adhesive cell interactions.

**Magnitude of Aggregation Forces.** The predictions of Fig. 1 show that a suspension of modeled RBCs captures the viscosity of healthy whole blood with cell aggregation. The plausibility of the aggregation strength was checked by calculation of the maximum force needed to break up two aggregated RBCs (see *SI Text*). The breakup pulling force in the normal direction is about 3.0–7 pN, where the lower value corresponds to a peeling breakup. Tangential or sliding breakup requires a force in the range of 1.5–3 pN. These forces are much smaller than those imposed on single RBCs in stretching tests with optical tweezers (35), and they are consistent with observations of rouleaux, which do not show any large cell deformations. In addition, measurements of a disaggregation force in shear flow by Chien et al. (36) indicate that the shear stress required to break up a rouleaux structure lies approximately between 0.01 and 0.1 Pa, whereas the analogous simulations with the MS-RBC model yield about 0.02 Pa (see *SI Text*).

**Conclusions.** The accurate prediction of the non-Newtonian behavior from simulations of cell suspensions suggests a new paradigm for rheology of cell suspensions and blood in particular. As an example, an abnormal increase in RBC aggregation is a pathological state associated with many diseases, such as deep venous thrombosis, atherosclerosis, AIDS, myeloma, and diabetes mellitus, which may afflict many different sites of the human arterial tree (37–40). However, such correlations have had few theoretical guidelines for their interpretation. The modeling of cells whose parameters are determined from experiments on single cells (41) can be extended to abnormal and diseased cells, and in combination with the aggregation model, their suspensions can be simulated to allow quantitative comparison with rheological measurements and to guide in vivo ultrasonic measurements to yield a more precise diagnosis of the aforementioned diseases (40). The predictive capability of accurate modeling of cell and capsule suspensions can be readily extended to a variety of engineering and material science applications. Such simulations may aid in the development of new soft materials and may drive the tuning process and optimization of their properties.

## Methods

**Simulation Method.** The DPD method (20, 21) is a particle-based mesoscopic simulation technique. A DPD system is represented by  $N$  point particles, which interact through pairwise soft potentials and move according to the Newton's second law of motion; see also *SI Text*.

**RBC Models.** An MS-RBC (15) is constructed by a collection of discrete points (500 in this work), which are the vertices of a triangular network of springs with a "dashpot" on the membrane surface. The network assumes fixed connectivity and supplies the elastic and the viscous response of an RBC membrane. To mimic membrane bending rigidity, a bending resistance is implemented between all neighboring triangular plaquettes. In addition, area and volume constraints are enforced to model incompressibility of an RBC membrane and the cytosol, respectively. The LD-RBC model (23) is constructed as a closed torus-like ring of 10 overlapping colloidal particles connected by springs. Each colloidal particle is represented by a single DPD particle with a repulsive core. A bending resistance between two neighboring springs is also incorporated. More details on the RBC models can be found in the *SI Text*.

**Aggregation Models.** For a blood suspension, the attractive cell–cell interactions are crucial for simulation of aggregation into rouleaux. These forces are approximated using the Morse potential  $U(r) = D_e[e^{2\beta(r_0-r)} - 2e^{\beta(r_0-r)}]$ , where  $r$  is the separation distance,  $r_0$  is the zero force distance,  $D_e$  is the well depth of the potential, and  $\beta$  characterizes the interaction range. For the MS-RBC model, the Morse potential interactions are implemented between every pair of vertices of separate RBCs if they lie within a defined potential cutoff radius. For the LD-RBC model, the aggregation force acts between centers of mass of different RBCs if the cells are properly aligned. Thus, the Morse potential is applied only if the angle between the normals of two cells does not exceed a critical angle. The aggregation forces for blood were calibrated for a single shear rate and no further adjustments were made in the subsequent computation of suspension viscosity. More details on the aggregation models can be found in the *SI Text*.

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