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*Biomechanics of red blood cells in human spleen
and consequences for physiology and disease*

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Correction

BIOPHYSICS AND COMPUTATIONAL BIOLOGY, ENGINEERING

Correction for “Biomechanics of red blood cells in human spleen and consequences for physiology and disease,” by Igor V. Pivkin, Zhangli Peng, George E. Karniadakis, Pierre A. Buffet, Ming Dao, and Subra Suresh, which appeared in issue 28, July 12, 2016, of *Proc Natl Acad Sci USA* (113:7804–7809; first published June 27, 2016; 10.1073/pnas.1606751113).

The authors note that on page 7806, the expression for red blood cell (RBC) volume (Eq. 3) appeared incorrectly. The correct exact expression for RBC volume, to be taken within Eq. 8 corresponding to the dotted line in Fig. 3, should be given as

$$V = 2 \left[\frac{4}{3} \pi R^3 - \frac{1}{3} \pi h^2 (3R - h) \right] + \frac{\pi L_s}{2} \left(R + \frac{L_s}{2} \right) \cos \theta (R \sin 2\theta - L_s \theta) + \frac{\pi}{4} \left(L_s^3 \sin \theta - \frac{L_s^3 \sin^3 \theta}{3} \right) \quad [3]$$

In this case, there is no longer a need for Eqs. 5 and 6. The dotted curve in Fig. 3 was actually plotted with the exact analytical solution using the correct Eq. 3 above for the RBC volume. All results presented originally in Fig. 3 are correct with the above Eq. 3.

The authors further note that the originally printed expression for RBC volume (Eq. 3) on page 7806, in conjunction with the original Eqs. 5 and 6, should constitute an approximate solution for RBC volume. After correcting a typo, the correct approximate expression for RBC volume (Eq. 3) would appear as $V = 2 \left[\frac{4}{3} \pi R^3 - \frac{1}{3} \pi h^2 (3R - h) \right] + 2\pi A_{vc}$. When taking this corrected approximate solution for RBC volume in Eq. 8, the (approximate) analytical solution would not match exactly (i.e., being slightly lower than) the dotted curve shown in Fig. 3.

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Biomechanics of red blood cells in human spleen and consequences for physiology and disease

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Contributed by Subra Suresh, April 30, 2016 (sent for review February 13, 2016; reviewed by Rafi Ahmed and Gang Bao)

Red blood cells (RBCs) can be cleared from circulation when alterations in their size, shape, and deformability are detected. This function is modulated by the spleen-specific structure of the interendothelial slit (IES). Here, we present a unique physiological framework for development of prognostic markers in RBC diseases by quantifying biophysical limits for RBCs to pass through the IES, using computational simulations based on dissipative particle dynamics. The results show that the spleen selects RBCs for continued circulation based on their geometry, consistent with prior in vivo observations. A companion analysis provides critical bounds relating surface area and volume for healthy RBCs beyond which the RBCs fail the “physical fitness test” to pass through the IES, supporting independent experiments. Our results suggest that the spleen plays an important role in determining distributions of size and shape of healthy RBCs. Because mechanical retention of infected RBC impacts malaria pathogenesis, we studied key biophysical parameters for RBCs infected with *Plasmodium falciparum* as they cross the IES. In agreement with experimental results, surface area loss of an infected RBC is found to be a more important determinant of splenic retention than its membrane stiffness. The simulations provide insights into the effects of pressure gradient across the IES on RBC retention. By providing quantitative biophysical limits for RBCs to pass through the IES, the narrowest circulatory bottleneck in the spleen, our results offer a broad approach for developing quantitative markers for diseases such as hereditary spherocytosis, thalassemia, and malaria.

erythrocytes | microcirculation | spleen clearance | malaria | spherocytosis

The spleen, about 10–12 cm in length with a mass of 100–200 g and located in the left superior abdomen, is the largest lymphatic organ in the human body (1, 2). The spleen plays a key role in the human immune system by protecting the body from pathogenic microorganisms reaching the bloodstream, through innate phagocytosis or adaptive responses operated by lymphocytes and antibodies. The spleen also serves as a filter that can remove red blood cells (RBCs) from circulation because of either physiological senescence or pathological alterations.

RBCs can be recognized as altered when changes in their shape, size, or surface are detected or their deformability is impaired as a consequence of such changes. Archetypical surface alterations are externalization of phosphatidyl-serine on the outer leaflet of the phospholipid bilayer of the RBC membrane or fixation of antibody on surface antigens, as occurs in transfusion mismatch. Sensing of these alterations by macrophages can occur all over the body with strong predominance in the spleen and liver (3). By contrast, recognition of altered size, shape, and deformability is considered a spleen-specific function. Surface area loss and reduced deformability also occur during aging of healthy RBCs (4). The archetypical disease where mechanical retention of RBCs in the spleen is the central pathogenic process is hereditary spherocytosis (HS). HS is a genetic disorder resulting in dysfunctional membrane proteins that play a role in transforming the shape of the RBC from a normal discocyte to a sphere. HS occurs at a frequency of 1 in 5,000 births in the Caucasian population and is the most common origin

of hereditary intravascular and extravascular hemolysis. In HS, defects in band 3, ankyrin and spectrin membrane proteins connecting the RBC membrane to the spectrin network can lead to the vesiculation of unsupported lipid bilayer. Such vesiculation causes a gradual reduction in cell surface area by as much as 20% compared with that of a healthy RBC (5). This reduction, in turn, can significantly increase the retention rates of RBCs in the spleen because of their increased sphericity (6). Severe reduction in RBC deformability attributable to spherocytosis results in the flow of RBCs to be obstructed as they pass through the spleen (7, 8). Consequently, the RBCs are phagocytosed causing hemolytic anemia and splenomegaly (i.e., enlarged spleen). The fact that surgical removal of the spleen (splenectomy) alleviates anemia to a large extent in patients with severe HS lends support to the argument that recognition of altered deformability of RBCs is a specific function of the spleen. Increased in vitro mechanical retention of RBCs collected in splenectomized subjects further supports this inference (9). RBC deformability is impaired in several other conditions including thalassemia (10), burns (11), and *Plasmodium falciparum* malaria (12). During its 48-h life cycle, the *P. falciparum*-infected RBC progressively increases in stiffness. During the so-called “ring” stage (i.e., within 24 h of host cell invasion by the parasite), the infected RBCs (iRBCs) undergo ~9.6% surface area loss (13) and reduced deformability arising from up to a fourfold increase in membrane shear modulus (14). Innate mechanical retention of a proportion of normally circulating ring-iRBCs has been observed ex vivo in a

Significance

The 3D opening of the interendothelial slit in human spleen creates a physical fitness test for red blood cells (RBCs) and clears them from circulation if their geometry and deformability are altered. We present a unique computational framework for the development of prognostic markers for diseases that alter RBC physical characteristics and identify quantitative limits for splenic slit clearance. Our work shows how the splenic slit determines distributions of size and shape of healthy RBCs. Our work lays the groundwork for systematic reconstruction of RBC navigation in the human spleen with consequences for a variety of acute and chronic medical conditions associated with hereditary alterations of RBCs, infectious diseases, and cancers.

Author contributions: I.V.P., Z.P., M.D., and S.S. designed research; I.V.P. and Z.P. performed research; I.V.P., Z.P., G.E.K., P.A.B., M.D., and S.S. analyzed data; and I.V.P., Z.P., G.E.K., P.A.B., M.D., and S.S. wrote the paper.

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model that provides the overall trends and bounds for comparison with computations and experiments so as to determine how the spleen influences the size and shape distributions of RBCs.

Results and Discussion

Computational Framework. We use the DPD-based RBC model (25, 26) for simulations. The details can be found in *Methods* and *SI Text*.

Fig. 1A is a schematic illustration of a venous sinus located in the cords in the red pulp of the human spleen. The sinuses comprise aligned endothelial cells that are connected by stress fibers to annular fibers. From such geometrical considerations and from detailed structural studies of the sinus wall in the TEM (20), we constructed the computational model for RBC traversal through the IES (Fig. 1B). The average height of the slits, $H_s \sim 0.65 \mu\text{m}$ with a range from 0.25 to 1.2 μm and an SD of 0.5 μm , and average length (the thickness of the slit wall) $L_s \sim 1.89 \mu\text{m}$ with a range from 0.9 to 3.2 μm and an SD of 0.9 μm . The width of the slit $W_s \sim 2\text{--}3 \mu\text{m}$ and the stress fiber width $W_f \sim 1 \mu\text{m}$ (27). Unless otherwise noted, we use the following reference geometry of the IES for all simulations in this study: height $H_s = 1.2 \mu\text{m}$, width $W_s = 4.0 \mu\text{m}$, and length $L_s = 1.89 \mu\text{m}$. The stress fiber width is $W_f = 1 \mu\text{m}$. These geometrical parameters were chosen from the upper bound value for slit height from TEM ultrastructural characterization experiments (20) for two reasons. First, because of the observation angle, the IES dimensions measured experimentally might have been underestimated. Second, the actual IES opening shape is not a rectangle with sharp corners. The opening is likely closer to an elongated ellipse, and the cross-section observed in the experiment might not exceed the maximum dimension in the height direction. Note that the effective stiffness of endothelial cells is ~ 100 times higher than that of RBCs (28); the slit walls were thus modeled as rigid side walls of circular cylinders, comprising endothelial cells, and thin slabs, representing annular stress fibers (Fig. 1B).

Analytical Framework. A simplified axisymmetric model amenable to theoretical analysis is first considered here (Fig. 2) to elucidate the effects of geometric constraints on RBC-IES interactions, which are further taken up for much more detailed investigation in our computational simulations that relax many of these geometric constraints.

We assume that the slit cross-section in the y - z plane is circular (Fig. 2) instead of rectangular (Fig. 1B), but with the same cross-sectional area such that

$$D_s = 2\sqrt{H_s W_s / \pi}. \quad [1]$$

The 3D slit geometry (Fig. 1B) is therefore approximated by the surface of a torus (Fig. 2). The deformed RBC at the critical condition consists of three parts: two spherical balls connected by a central connecting part (torus). The area and volume of the RBC are given, respectively, as

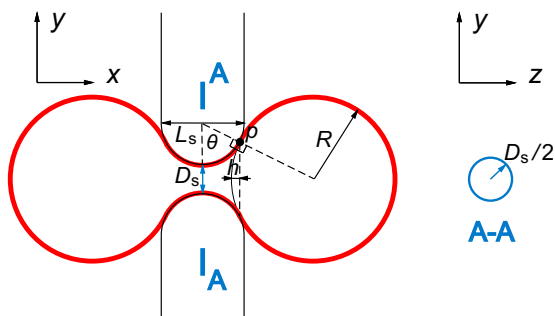


Fig. 2. Schematic illustration of the limiting geometry considered in the theoretical framework. The red solid line represents the RBC. At point p , the RBC surface is tangential to the surfaces of the endothelial cells. The cross-section A-A is shown on the right, where the solid line circle represents the slit cross-section. We approximate the real rectangular slit with the circular slit with the same cross-sectional area.

$$A = 4(2\pi R^2 - \pi R h) + 2\pi L_s \theta \left(\frac{D_s}{2} + \frac{L_s}{2} - \frac{L_s \sin \theta}{2\theta} \right) \quad [2]$$

and

$$V = 2 \left[\frac{4}{3} \pi R^3 - \frac{4}{3} \pi h^2 (3R - h) \right] + 2\pi A_c y_c, \quad [3]$$

where

$$h = R - R \sqrt{1 - \frac{\left(\frac{D_s}{2} + \frac{L_s}{2}\right)^2}{\left(R + \frac{L_s}{2}\right)^2}} \quad [4]$$

is shown in Fig. 2, which is the height of spherical cap cut by the y - z plane intersecting point p ,

$$y_c = \frac{D_s}{2} + \frac{L_s}{2} - \frac{L_s \sin \theta}{3\theta} \quad [5]$$

is the y coordinate of the centroid of the upper one-half cross-section of the torus part,

$$A_c = \frac{L_s \sin \theta}{2} \left[D_s + L_s - L_s \left(\frac{D_s}{2} + \frac{L_s}{2} \right) / \left(R + \frac{L_s}{2} \right) \right] - \frac{L_s^2}{2} (2\theta - \sin 2\theta) \quad [6]$$

is one-half of the cross-section area of the torus part in the x - y plane, and

$$\theta = \arccos \left[\left(\frac{D_s}{2} + \frac{L_s}{2} \right) / \left(R + \frac{L_s}{2} \right) \right] \quad [7]$$

is an angle of the torus part as shown in Fig. 2. The area and volume of the middle part are calculated based on Pappus's centroid theorem. Given the radius of the slit opening, D_s , and the thickness of the sinus wall, L_s , the axisymmetric theory thus provides the maximum volume at which the RBC with fixed surface area will be able to cross the slit of a given geometry. If Eq. 3 is rewritten as $V = f(R)$ and its inverse function is given by $R = f^{-1}(V)$, Eq. 4 and Eq. 7 can be expressed as $h = h(R) = h(f^{-1}(V)) = g_1(V)$ and $\theta = \theta(R) = \theta(f^{-1}(V)) = g_2(V)$. The relationship between the surface area A and the volume V is given by

$$A = 4 \left\{ 2\pi [f^{-1}(V)]^2 - \pi f^{-1}(V) g_1(V) \right\} + 2\pi L_s g_2(V) \left\{ \frac{D_s}{2} + \frac{L_s}{2} - \frac{L_s \sin[g_2(V)]}{2g_2(V)} \right\}. \quad [8]$$

Comparison with Experiments. The bounds predicted by the foregoing analysis can be compared with experimental observations in the literature (23) as well as with our computational simulations. These comparisons benefit from further insights gained through ex vivo experiments performed on isolated perfused human spleen (16) as well as on a synthetic spleen comprising a microsphere filtration system (20). The latter work suggests that the geometric characteristics obtained from spleen experiments could be recapitulated using micrometer-sized metal spheres. The microsphere filtration device was designed based on the observed slit shape and dimensions, with a 5-mm-thick layer of mixture of spheres, 5 to 15 μm in diameter. The pressure difference in the experiments where the RBC suspension was pushed through the device was held at 63.2 cm H_2O , giving an average pressure gradient of 1 Pa/ μm inside the filter (20). These experiments reveal that RBCs with

Taken together, these results provide a robustly validated quantitative framework on how the human spleen plays its bio-mechanical filtering role that contributes to the quality assurance of circulating RBCs. This framework opens unique pathways for the use of quantitative morphological features of circulating RBC—now rapidly obtained by imaging flow cytometry (6, 37)—as markers of hyposplenism in patients with suspected inherited or acquired splenic dysfunction, a condition associated with severe infectious, circulatory, and proliferative complications (38). In addition to splenectomized patients (38), hyposplenism affects a majority of children with sickle-cell disease (39) and patients suffering from an array of acute or chronic medical conditions (40), such as celiac disease, inflammatory bowel diseases, cirrhosis, and viral or bacterial infections. More precise estimates of splenic function would guide patient management for an optimal adaptation of antimicrobial prophylaxis (41) and prevention of thrombotic events (42). Several conditions, such as malaria, thalassemia, or severe hematological diseases (leukemia and lymphomas), induce enlargement of the spleen with subsequent propensity to filter not only abnormal cells but also healthy RBCs (43), thus causing anemia. Our approach forms the first step of a potential systematic reconstruction of the navigation of RBCs in the human spleen across narrow slits but also upstream and downstream from this unique circulatory bottleneck.

Methods

RBC Model. The details of the DPD based RBC model can be found in *SI Text, RBC Model*.

Ex Vivo and Synthetic Spleen Studies and Structural Characterization. Clinical interventions associated with left splenopancreatectomy for benign tumors

of the pancreas (16) have led to ex vivo experiments in isolated human spleen perfused with healthy RBCs, iRBCs invaded by *P. falciparum*, as well as chemically treated RBCs with controlled surface area loss (6, 13, 16, 20). Geometric characteristics of RBC interactions and trapping from ex vivo human spleen have also been replicated using a “synthetic spleen” made of metal spheres (20). These studies showed that the retention of abnormal RBCs was influenced predominantly by their mechanical properties and that these effects could occur in the absence of ligand–receptor interactions between RBCs and the relevant splenic structures. Additional discussions are included in *SI Text, Ex Vivo and Synthetic Spleen Studies and Structural Characterization*.

DPD Method. The DPD method invokes a coarse-grained molecular dynamics approach, further details of which are described in *SI Text, DPD Model and Scaling of Physical Units*.

Simulation Setup. The simulation setup is illustrated in Fig. 1B. More details and input parameters can be found in *SI Text, Simulation Setup*.

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- Mebius RE, Kraal G (2005) Structure and function of the spleen. *Nat Rev Immunol* 5(8):606–616.
- Petroianu A (2011) *The Spleen* (Bentham Science Publishers, Hilversum, The Netherlands).
- Jandl JH, Greenberg MS, Yonemoto RH, Castle WB (1956) Clinical determination of the sites of red cell sequestration in hemolytic anemias. *J Clin Invest* 35(8):842–867.
- Waugh RE, et al. (1992) Rheologic properties of senescent erythrocytes: Loss of surface area and volume with red blood cell age. *Blood* 79(5):1351–1358.
- Suresh S (2006) Mechanical response of human red blood cells in health and disease: Some structure-property-function relationships. *J Mater Res* 21(8):1871–1877.
- Safeukui I, et al. (2012) Quantitative assessment of sensing and sequestration of spherocytic erythrocytes by the human spleen. *Blood* 120(2):424–430.
- Kumar V, Abbas AK, Aster JC (2012) *Robbins Basic Pathology* (Elsevier, Saunders, Philadelphia), 9th Ed.
- Perrotta S, Gallagher PG, Mohandas N (2008) Hereditary spherocytosis. *Lancet* 372(9647):1411–1426.
- Prendki V, et al. (2012) Reduced deformability of circulating erythrocytes: A marker of hyposplenism. *Am J Hematol* 87(10):E81–E82.
- Dondorp AM, et al. (1999) Red cell deformability, splenic function and anaemia in thalassaemia. *Br J Haematol* 105(2):505–508.
- Zaets SB, et al. (2003) Burn-induced red blood cell deformability and shape changes are modulated by sex hormones. *Am J Surg* 186(5):540–546.
- Cranston HA, et al. (1984) Plasmodium falciparum maturation abolishes physiologic red cell deformability. *Science* 223(4634):400–403.
- Safeukui I, et al. (2013) Surface area loss and increased sphericity account for the splenic entrapment of subpopulations of Plasmodium falciparum ring-infected erythrocytes. *PLoS One* 8(3):e60150.
- Mills JP, et al. (2007) Effect of plasmodial RESA protein on deformability of human red blood cells harboring Plasmodium falciparum. *Proc Natl Acad Sci USA* 104(22):9213–9217.
- Safeukui I, et al. (2008) Retention of Plasmodium falciparum ring-infected erythrocytes in the slow, open microcirculation of the human spleen. *Blood* 112(6):2520–2528.
- Buffet PA, et al. (2006) Ex vivo perfusion of human spleens maintains clearing and processing functions. *Blood* 107(9):3745–3752.
- Buffet PA, Safeukui I, Milon G, Mercereau-Puijalon O, David PH (2009) Retention of erythrocytes in the spleen: A double-edged process in human malaria. *Curr Opin Hematol* 16(3):157–164.
- Buffet PA, et al. (2011) The pathogenesis of Plasmodium falciparum malaria in humans: Insights from splenic physiology. *Blood* 117(2):381–392.
- Duez J, et al. (2015) Mechanical clearance of red blood cells by the human spleen: Potential therapeutic applications of a biomimetic RBC filtration method. *Transfus Clin Biol* 22(3):151–157.
- Deplaine G, et al. (2011) The sensing of poorly deformable red blood cells by the human spleen can be mimicked in vitro. *Blood* 117(8):e88–e95.
- Groom AC, Schmidt EE, MacDonald IC (1991) Microcirculatory pathways and blood flow in spleen: New insights from washout kinetics, corrosion casts, and quantitative intravital videomicroscopy. *Scanning Microsc* 5(1):159–173.
- Freund JB (2013) The flow of red blood cells through a narrow spleen-like slit. *Phys Fluids* 25(11):110807.
- Canham PB, Burton AC (1968) Distribution of size and shape in populations of normal human red cells. *Circ Res* 22(3):405–422.
- Hoogerbrugge PJ, Koelman J (1992) Simulating microscopic hydrodynamic phenomena with dissipative particle dynamics. *Europhys Lett* 19(3):155–160.
- Pivkin IV, Karniadakis GE (2008) Accurate coarse-grained modeling of red blood cells. *Phys Rev Lett* 101(11):118105.
- Peng Z, et al. (2013) Lipid bilayer and cytoskeletal interactions in a red blood cell. *Proc Natl Acad Sci USA* 110(33):13356–13361.
- Chen LT, Weiss L (1973) The role of the sinus wall in the passage of erythrocytes through the spleen. *Blood* 41(4):529–537.
- Hochmuth RM (2000) Micropipette aspiration of living cells. *J Biomech* 33(1):15–22.
- Gifford SC, et al. (2003) Parallel microchannel-based measurements of individual erythrocyte areas and volumes. *Biophys J* 84(1):623–633.
- Sens P, Gov N (2007) Force balance and membrane shedding at the red-blood-cell surface. *Phys Rev Lett* 98(1):018102.
- Park Y, et al. (2008) Refractive index maps and membrane dynamics of human red blood cells parasitized by Plasmodium falciparum. *Proc Natl Acad Sci USA* 105(37):13730–13735.
- Bow H, et al. (2011) A microfabricated deformability-based flow cytometer with application to malaria. *Lab Chip* 11(6):1065–1073.
- Quinn DJ, et al. (2011) Combined simulation and experimental study of large deformation of red blood cells in microfluidic systems. *Ann Biomed Eng* 39(3):1041–1050.
- Evans EA, Waugh R, Melnik L (1976) Elastic area compressibility modulus of red cell membrane. *Biophys J* 16(6):585–595.
- Dondorp AM, et al. (2008) Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. *J Infect Dis* 197(1):79–84.
- Guest MM, Bond TP, Cooper RG, Derrick JR (1963) Red blood cells - Change in shape in capillaries. *Science* 142(3597):1319–1321.
- Jauréguiberry S, et al.; French Artesunate Working Group (2014) Postartesunate delayed hemolysis is a predictable event related to the lifesaving effect of artemisinins. *Blood* 124(2):167–175.
- Kristinsson SY, Gridley G, Hoover RN, Check D, Landgren O (2014) Long-term risks after splenectomy among 8,149 cancer-free American veterans: A cohort study with up to 27 years follow-up. *Haematologica* 99(2):392–398.
- Rogers ZR, et al.; BABY HUG (2011) Biomarkers of splenic function in infants with sickle cell anemia: Baseline data from the BABY HUG Trial. *Blood* 117(9):2614–2617.
- Di Sabatino A, Carsetti R, Corazza GR (2011) Post-splenectomy and hyposplenic states. *Lancet* 378(9785):86–97.
- Lammers AJJ, et al. (2012) Hyposplenism: Comparison of different methods for determining splenic function. *Am J Hematol* 87(5):484–489.
- Crary SE, Buchanan GR (2009) Vascular complications after splenectomy for hematologic disorders. *Blood* 114(14):2861–2868.
- Looareesuwan S, et al. (1987) Dynamic alteration in splenic function during acute falciparum malaria. *N Engl J Med* 317(11):675–679.