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Optical measurement of biomechanical properties of individual erythrocytes from a sickle cell patient

HeeSu Byun [a,1], Timothy R. Hillman [b,1], John M. Higgins [c,d], Monica Diez-Silva [e], Zhangli Peng [e], Ming Dao [e,*], Ramachandra R. Dasari [b], Subra Suresh [e], YongKeun Park [a,*]

[a] Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea
[b] George R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[c] Center for Systems Biology and Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA
[d] Department of Systems Biology, Harvard Medical School, Boston, MA 02114, USA
[e] Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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ABSTRACT

Sickle cell disease (SCD) is characterized by the abnormal deformation of red blood cells (RBCs) in the deoxygenated condition, as their elongated shape leads to compromised circulation. The pathophysiology of SCD is influenced by both the biomechanical properties of RBCs and their hemodynamic properties in the microvasculature. A major challenge in the study of SCD involves accurate characterization of the biomechanical properties of individual RBCs with minimum sample perturbation. Here we report the biomechanical properties of individual RBCs from a SCD patient using a non-invasive laser interferometric technique. We optically measure the dynamic membrane fluctuations of RBCs. The measurements are analyzed with a previously validated membrane model to retrieve key mechanical properties of the cells: bending modulus; shear modulus; area expansion modulus; and cytoplasmic viscosity. We find that high cytoplasmic viscosity at ambient oxygen concentration is principally responsible for the significantly decreased dynamic membrane fluctuations in RBCs with SCD, and that the mechanical properties of the membrane cortex of irreversibly sickled cells (ISCs) are different from those of the other types of RBCs in SCD.

1. Introduction

Sickle cell disease (SCD) or sickle cell anemia is an inherited autosomal blood disorder characterized by abnormal mechanical and rheological behavior of red blood cells (RBCs). SCD is caused by sickle hemoglobin (HbS), a variant hemoglobin (Hb) molecule resulting from a point mutation in the β-globin gene [1]. Upon deoxygenation, HbS polymerizes or self-assembles inside the RBC and significantly alters and damages the cytoskeleton and membrane cortex, resulting in a sickle-shaped RBC. This sickle RBC has decreased deformability, causing abnormal rheology in sickle blood and eventually various complications of SCD: ischemia and organ damage can result when microcirculation is impeded due to the poorly deformable RBCs. As a result of these complications and the limited choices for medical treatments, life expectancy for SCD patients is short; only 50% of patients with SCD survive beyond their fifth decade [2].

Characterization of the mechanical properties of RBCs is crucial to understanding the pathophysiology of many RBC-related diseases [3–5]. While the biochemistry of HbS is well understood, the mechanical properties of individual RBCs in SCD have not been fully assessed, largely due to the limitations of the measurement techniques [6]. Studies using filtration [7] or ektacytometry [8] have revealed that the sickle RBCs are stiffer than normal RBCs. However, these techniques cannot distinguish the mechanical properties of subpopulations of sickle RBCs or isolated RBCs, and they measure properties averaged over all RBCs in a blood sample. Micropipette aspiration [9], optical tweezers [10], the parallel-plate flow chamber method [11], and atomic force microscopy [12] have been employed to study the biomechanics of SCD at the cellular level. Although these methods have significantly enhanced our understanding of sickle cell biomechanics, none of them can probe all of the key mechanical parameters of individual RBCs simultaneously. Moreover, these previous methods rely on large, quasi-static external loads or perturbations to deform sickle RBCs through physical contact, and thus are not well suited to measure mechanical properties within linear deformation regimes.

* Corresponding authors. Tel.: +82 42 350 2514 (Y. Park).
E-mail addresses: mingdao@mit.edu (M. Dao), yk.park@kasit.ac.kr (Y. Park).
[1] These authors contributed equally to this work.

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Here we non-invasively investigate the biomechanical properties of individual RBCs in SCD by measuring the dynamics of membrane fluctuations in the sickle RBCs. Dynamic cell membrane fluctuations, consisting of nanometer-scale displacement of the cell membrane with millisecond temporal resolution, of the RBCs are strongly correlated with the structures of the cell membrane cortex and can be altered by biochemical changes [13–15]. To quantitatively measure the dynamics of membrane fluctuations for individual RBCs, we use an optical interferometric technique [13]. We analyzed the measured membrane fluctuations using a composite membrane model [13] in order to simultaneously retrieve four key mechanical properties of RBCs: bending modulus κ; shear modulus μ; area expansion modulus K_A; and cytoplasmic viscosity η.

2. Materials and methods

2.1. RBC sample preparation

Blood specimens were collected during the normal course of patient care at Brigham and Women's Hospital, Boston, MA, and used in experiments in accordance with a research protocol approved by the Partners Healthcare Institutional Review Board (IRB protocol number: 2006P000066). Samples were obtained from both a healthy individual and an SCD patient. The SCD patient was under treatment with hydroxyurea. The blood was collected in EDTA anticoagulant and stored at 4 °C. Clinical measurements of RBC volume and Hb concentration were made using an Advia 2120 automated hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL).

Upon measurement, the blood samples, diluted with PBS (1:5 volume fraction), were sandwiched between two cover glasses. The RBCs were gently placed on glass plates and remained stationary during the measurements. The time period of examination for one group of samples was ~30 min. For each individual RBC, 256 frames of interferograms were measured at 120 fps, which lasted ~2 s. All of the measurements were performed at room temperature.

2.2. Diffraction phase microscopy

The experimental setup for diffraction phase microscopy (DPM) is shown in Fig. 1. A diode-pumped solid-state laser (wavelength λ = 532 nm, 50 mW output power, CrystaLaser, Reno, NV) was used as an illumination source for an inverted microscope (IX71, Olympus America Inc., Center Valley, PA). The microscope was equipped with a 60× objective lens (UIS2 PlanApo 60×, 1.42 NA, Olympus America Inc., Center Valley, PA), which facilitates a diffraction-limited transverse resolution of ~400 nm. A transmission grating (#46-072, Edmund Optics Inc. USA, 92 grooves mm^{-1}) was used to construct a common-path interferometry. With the additional optics used outside the microscope, the overall magnification of the DPM system was ~300×. A CMOS camera (FASTCAM 1024 PCI, Photron USA, Inc., San Diego, CA) was used to record interferograms.

2.3. Composite model of RBC

In order to retrieve key mechanical properties of RBCs from the measured membrane dynamic fluctuations, we used a continuum model of the composite spectrin-network/lipid membrane [16]. This model, incorporating the coupling between the bending and compression modes of the curved membrane, allows us to quantitatively determine the mechanical parameters of the cells. It has been experimentally validated with RBCs of different morphologies [13] and under different osmotic pressures [17]. The lipid bilayer, 4–5 nm thick, in the RBC membrane cortex can be mechanically characterized by a bending modulus κ and an area compression modulus K_A. On the cytoplasmic side of the membrane, the two-dimensional (2-D) triangular spectrin network is anchored to the bilayer via transmembrane proteins [18]. The mechanics of this spectrin network can be described as a 2-D elastic continuum having two equal Lamé constants, $\mu = \lambda$. The RBC cytosol primarily consists of Hb solution with viscosity η_c. Due to the high Hb concentration, η_c is significantly greater than the viscosity of the surrounding solvent, η_s. Thus, the mechanical description of the RBC model contains four important parameters, those identified in Section 1: κ, K_A, μ, η_c.

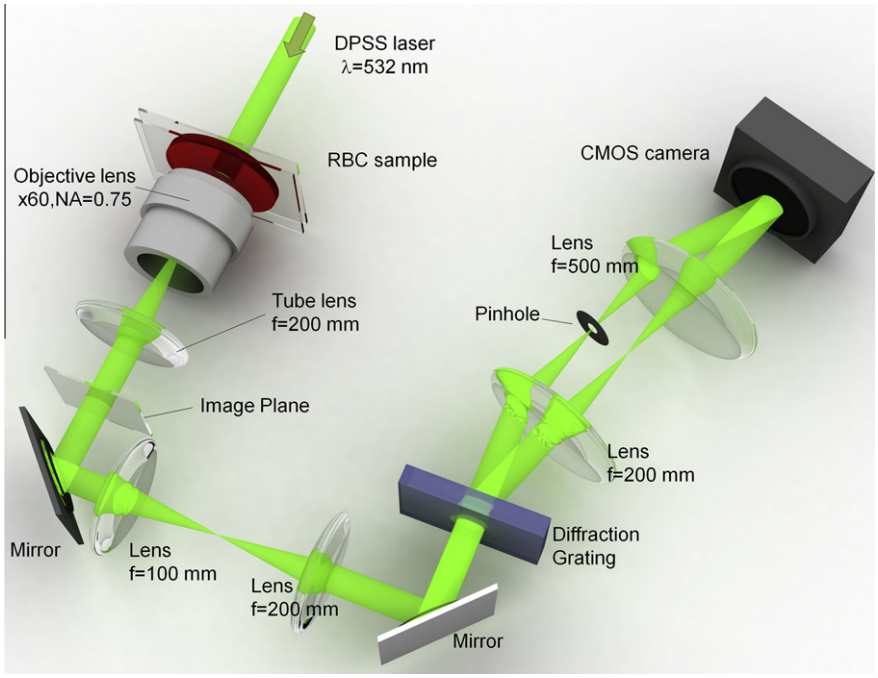

Fig. 1. Experimental setup of DPM.