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Cellular Biophysical Markers of Hydroxyurea Treatment in Sickle Cell Disease

Peter T. C. So^{1,2,3}, Poorya Hosseini,^{1,2,3} Sabia Z. Abidi,⁴ E. Du,⁵ Dimitrios P. Papageorgiou,⁴ YongKeun Park⁶, John Higgins,⁷ Gregory J. Kato,⁸ Subra Suresh,⁹ Ming Dao,^{4*} Zahid Yaqoob^{1*}

¹Laser Biomedical Research Center, Massachusetts Institute of Technology, Cambridge, MA 02139

²Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

³Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

⁴Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

⁵Department of Ocean and Mechanical Engineering, Florida Atlantic University, Boca Raton, FL 33431

⁶Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

⁷Department of Systems Biology, Harvard Medical School, Boston, MA 02115

⁸Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh, Pittsburgh, PA 15261

⁹Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA 15213

Summary: Hydroxyurea (HU) has been used clinically to reduce the frequency of painful crisis and the need for blood transfusion in sickle cell disease (SCD) patients. However, the mechanisms underlying such beneficial effects of HU treatment are still not fully understood. Studies have indicated a weak correlation between clinical outcome and molecular markers, and the scientific quest to develop companion biophysical markers have mostly targeted studies of blood properties under hypoxia. Using a common-path interferometric technique, we measure biomechanical and morphological properties of individual red blood cells in SCD patients as a function of cell density, and investigate the correlation of these biophysical properties with drug intake as well as other clinically measured parameters. Our results show that patient-specific HU effects on the cellular biophysical properties are detectable at normoxia, and that these properties are strongly correlated with the clinically measured mean cellular volume rather than fetal hemoglobin level.

Fig. 1. Optical measurement of morphological properties of RBCs. Morphological information about the sample (eccentricity, surface area and volume) are obtained from deformations of the optical wavefront after its traversal through RBCs. The phase delay of the optical wave front after passing through the sample is related to optical properties and morphology of the sample and can be accurately measured through advanced interferometry. Accurate measurements of the morphology over time reveal thermal fluctuations that cause out-of-plane displacements of the cell membrane (bottom right image), which provide insights into the biomechanics of the whole cell (bending and shear modulus as well the cytosol viscosity). Scale bar is 4

Sickle cell disease, including the most common form, sickle cell anemia, is an inherited blood disorder caused by a single point mutation in the β -globin gene (1). Sick cell hemoglobin (HbS) has the unique property of polymerizing when deoxygenated, triggering red blood cell (RBC) sickling and rigidification, leading to vaso-occlusion and impaired blood flow in capillaries and small vessels (2). Therefore, the pathophysiology of SCD is critically influenced by the oxygen partial pressure affecting the biomechanical properties of RBCs and their corresponding hemodynamic properties in microvasculature. In this work, we investigate the irreversible changes red cells undergo in the blood circulation in SCD patients at normal oxygen conditions. Figure 1 shows how morphological and biomechanical properties of the red cells are characterized through quantifying the deformations of the wavefront after passing through the cell. Measurements of the cellular biophysical properties of

red cells using transmission-type quantitative phase instruments is affected by the refractive index differences of individual cells; and normally an average value is assumed for the whole population under study. Here, we have improved the accuracy of such measurements by breaking down blood samples of SCD patients into four density categories that allows for more accurate characterization of cellular biophysical properties, see Figure 2(a). We show how these biophysical properties change as a function of the cell density that is related to hemoglobin content in the cytosol, e.g. Figure 2(b). We additionally investigated various biophysical properties of all the density-sorted groups for patients who are on and off HU treatment and managed to identify beneficial effects of HU treatment even under normoxic conditions, e.g. Figure 3(a). All measurements from on- and off-drug populations were grouped together for each density category to study the overall effect of the drug treatment. For almost all properties and all density categories, a statistically significant difference was observed as a result of HU treatment. These results show that RBCs under HU treatment are softer on average regardless of their density. RBCs of patients under HU treatment exhibit a higher volume and a lower surface area to volume on average. There was no clear difference between average eccentricity value for lighter sickle cells, however, denser cells did exhibit a higher eccentricity ratio for patients off HU treatment.

There is a range of clinically measured parameters that provide insights on the status of SCD patients. These laboratory parameters are broadly derived from either cellular evaluation tests such as hematocrit and mean corpuscular volume (MCV), or from the molecular profile tests such as blood composition using gel-electrophoresis. Two well-known responses of HU treatment are the induction of HbF and increase in MCV due to the ability of the drug to induce stress erythropoiesis (3,4). To assess whether MCV and/or HbF can drive differences in biophysical properties at normoxia, we categorized the patient data into low and high MCV and low and high HbF populations. We observed a statistically significant difference for all properties except eccentricity ratio when the patient data are categorized into low and high MCV populations based on the median MCV value for all patients, suggesting MCV may be the variable driving the observed RBC biomechanical differences. Surprisingly, separation of low and high HbF populations based on the median HbF value for all patients did not reveal any statistically significant differences in the biophysical properties.

Figure 2. Density fractionation of RBCs from sickle patients and healthy donors. (a) Representative images of RBCs in each density fraction from blood sample of a sickle patient. I, II, III and IV represent density fractions that are 26.6, 30.2, 34.1, and 37.8 g.dL⁻¹, respectively. (b) Cell membrane fluctuations of a sickle cell patient as a function of density of the hemoglobin inside the cytosol.

Figure 3. (a) amplitude of the cell membrane fluctuations for “on” and “off” the drug patients, higher amplitude generally indicates softer membrane. **(b)** Cell membrane fluctuations of the erythrocytes of cell patients in our study as a function of density of the hemoglobin inside the cytosol. Left graph shows the patient data categorized based on low and high clinically measured mean cellular volume. Right graph shows the patient data categorized based on low and high levels of fetal hemoglobin in the patient blood. Density I, II, III and IV are 26, 30, 34 and 38 g.dL⁻¹, respectively.

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