Novel Optical Signature for Sickle Cell Trait Red Blood Cells

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Abstract: We identified unique optical signatures for sickle cell trait, a condition where heterozygous individuals are carriers for the hemoglobin allele that causes sickle cell anemia, by using wide-field interferometric microscopy.

1. Introduction

In humans, stable polymorphisms in the hemoglobin gene (HbA) underlie sickle cell anemia (SCA), a devastating and common heritable blood disorder that with thalassemia affects 350 million globally. SCA is incurable, emerging by 4 years of age and requiring life long management due to severe and lengthy pain crises and organ failure. People with SCA have shorter life spans and experience a constellation of symptoms stemming from blockage of blood flow due to the sickle cell shape of red blood cells (RBCs). Triggered in the venous blood supply by low oxygen tension, microvascular occlusion is associated with pain in the chest and joints, small stokes resulting in reduced cognition, ulcers in the lower legs, poor eyesight or blindness, chronic anemia and reduced organ function. Current treatments are blood transfusion or hydroxyurea (HU), which stimulates expression of fetal hemoglobin, supplemented with folic acid, pain medication and antibiotics. Targeted therapies are a critical clinical need. SCA is an autosomal recessive disorder stemming from well characterized mutations including hemoglobin S (HbS; β6 Glu to Val) and hemoglobin C (HbC; β6 Glu to Lys). Unlike homozygotes for HbS or HbC who develop SCA, people heterozygous for the Hb polymorphisms (HbAS or HbAC) are termed sickle cell trait (SCT) and show few if any adverse health effects of the magnitude of SCA, and the additional marked heterozygous advantage of resistance to parasitic infections including malaria. However, HbAC or HbAS individuals suffer increased risk for kidney disease, stroke, and illness or death from intense exercise. In the United States, approximately 100,000 people have SCA, and about two million people have SCT, which includes 8% of the African American population (NIH 2011 SS Report). SCT and SCA are prevalent in people of African and Mediterranean descent and in those from Central and South America, the Middle East and the Caribbean, where historically positive selection for SCA in humans evolved from the advantage of resistance to malaria.

At present, SCA and SCT are diagnosed using electrophoresis of hemoglobin, a relatively expensive laboratory-based technique. Our goal was to develop a low cost non-invasive optical signature for sickle cell trait to use for diagnosis of asymptomatic individuals with SCT in the field.

Wide-field digital interferometry (WFDI) is a noncontact technique that records the complex field (amplitude and phase) of the light that has interacted with the sample, where no exogenous labeling or special sample preparation are involved. Using the recorded interference pattern, one can obtain the quantitative phase profile of the sample representing the optical path delay map for each spatial point in the sample image. This map is dependent on both the integral refractive index of the sample and its thickness. Mature RBCs are an attractive model for WFDI since the cytoplasm has a relatively homogeneous refractive index structure and thus RBC thickness profiles can be directly obtained from their quantitative phase profile.

2. Methods and Results

Peripheral blood from homozygotes (SS), heterozygotes (AS), and healthy controls was collected into K-EDTA Vacutainer tubes (BD Biosciences) following consent in the Duke Sickle Cell Clinic according to IRB guidelines. 100 μl whole blood was washed twice in HBBS and attached for 10 min at 37^{O}C to optical glass that was pre-coated with human fibronectin (25 μg/ml in PBS; BD Biosciences). Attached RBCs were washed gently in HBBS diluted 1:1 with EBM2 containing EGM-2 MV SingleQuots (Invitrogen) before imaging at room temperature. WFDI measurements were performed using an interferometric microscopy setup, reported in detail in our previous publications [1]. In the optical setup, a coherent laser light (17 mW HeNe) is spatially filtered and split to reference and object beams. The object beam is transmitted through the sample and magnified by a microscope objective (40×, 0.66 numerical aperture). The reference beam passes through a similar compensating microscope objective and combined with the object beam at a small angle. A tube lens projects the combined fields on a digital camera 640 × 480, 7.4 μm × 7.4 μm pixels, 120 full frames per second), where an off-axis interferogram of the sample is

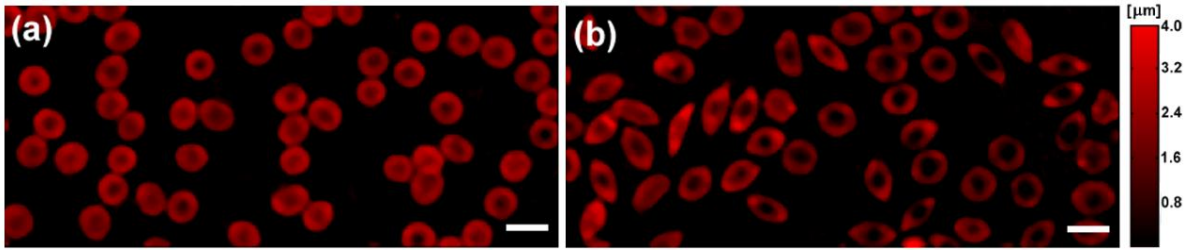

Fig. 1 WFDI-based thickness profiles of (a) healthy RBCs, (b) SCA RBCs, demonstrating the different RBC morphology that characterizes SCA. Scale bar represents 10 μm. Color bar represents thickness [1].

created. Using a single interferogram, the fully quantitative phase profile of the sample is obtained by a spatial filtering process followed by a quality-guided phase-unwrapping algorithm. Figure 1 presents the quantitative phase profile of the RBCs from a healthy person and from a person with SCA.

We then used WFDI to image RBCs from an HbS/HbS individual with SCA (SS) and two HbA/HbS individuals heterozygous for sickle cell trait (AS-1; AS-2) compared to a healthy HbA/HbA race and gender-matched control (AA). RBCs from SCT generally show identical morphology to healthy controls without obvious change in shape. Normalized average fluctuations from individual RBCs chosen randomly and independent of morphology from each group are plotted in Figure 2. We found that average fluctuations increase with presence of the sickle cell anemia allele, with heterozygotes (AS-1 and AS-2) showing an apparent *wider distribution* and higher values than healthy controls, and even higher fluctuations and a similar wider distribution of values in RBCs the sickle cell sample. We used Lilliefor's test to test if the fluctuations within each of the four groups were normally distributed. We found that AA showed a normal distribution, whereas AS-1, AS-2 and SS shared a non-normal distribution.

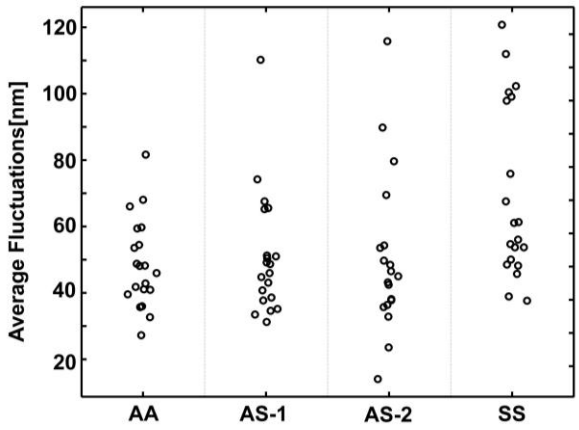

Fig. 2 Normalized average fluctuations in RBCs from a healthy control (AA), sickle cell trait (AS-1), a second sickle cell trait (AS-2), and sickle cell anemia (SS).

3. Conclusions

For the first time to our knowledge, we have applied WFDI to quantify nanometer-scale fluctuations in thickness and resulting stiffness profiles in RBCs from SCT individuals, asymptomatic carriers of the hemoglobin allele that causes SCA. We report a unique optical signature for SCT: a wide non-normal distribution of average membrane fluctuations distinct from SCA and healthy controls. Mechanistically, it is possible in SCT that changes to attachment of the actin cytoskeleton to the inner cell surface through polymerization of HbS or HbC alters membrane fluctuations. Alternatively, stochastic transient mass polymerization of hemoglobin may contribute to the optical signature. The WFDI-based optical signatures are sensitive enough to detect internal cytoplasmic changes which not visible from overall cell morphology, and thus have the potential to predict vaso-occlusive events or be used to score efficacy of targeted therapies.

References

1. N. T. Shaked, L. L. Satterwhite, G. A. Truskey, M. J. Telen, and A. Wax, "Quantitative microscopy and nanoscopy of sickle red blood cells performed by wide field digital interferometry," Journal of Biomedical Optics Letters 16, 030506:1-3, 2011.