CP2: Biomechanics of Sickle Cell Disease: Gregory Kato, MD (University of Pittsburgh Medical Center), Ming Dao (MIT), Bruce Tromberg (UC Irvine)

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Associated with: TRD2

Significance: Sickle cell disease (SCD) is a genetic blood disorder resulted from a single point mutation in the β-globin gene (1, 2). The mutation causes sickle hemogralin (HbS) to bind and form long chains when deoxygenated. The HbS polymerization is believed to decrease RBC deformability and trigger RBC sickling (Fig. 1), which leads to vaso-occlusion in capillaries and small venules (3, 4). Each year, over 180,000 children are born with SCD worldwide (5). In the US alone, 90,000 to 100,000 people – mostly African Americans – have SCD, and about 1 in 12 African Americans carry the sickle cell trait.

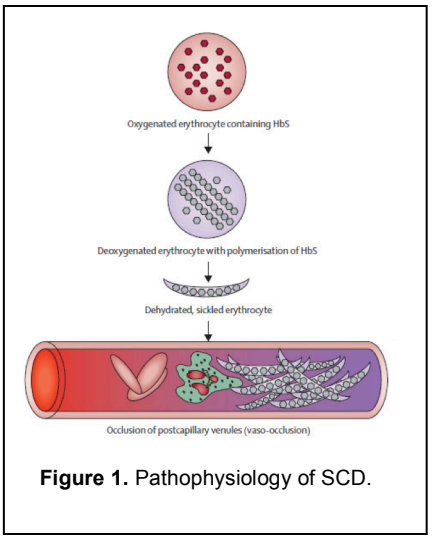

Figure 1. Pathophysiology of SCD.

Dr. Gregory Kato, Director of Adult Sickle Cell Center of Excellence at Pittsburgh, is a hematologist with an active translational research interest in SCD. He leads a vibrant clinical and laboratory bench research team that focuses on the causes of various forms of vascular dysfunction associated with SCD (6-22). Dr. Kato is also interested in understanding the mechanisms of existing therapies (23-28) as well as developing new treatments to treat accute syndromes of SCD or prevent these crisis altogether (29-34).

While the molecular details of the sickle hemoglobin (HbS) polymerization (35) as well as the clinical heterogeneity of SCD (36) are now reasonably well understood, the proper understanding of RBC deformability and shape changes when deoxygenated especially on individual cell basis remains elusive in SCD investigations primarily due to the lack of appropriate measurement techniques. The best attempts to understand sickle RBC mechanics have been made using micropipette aspiration (3, 37) at fully stabilized oxygenation / deoxygenation conditions. Other methods to quantify mechanical properties of sickle RBCs including atomic force microscopy (AFM) (38-41), optical and magnetic tweezers (42, 43), and electric field deformation (44) are limited to study fully stabilized RBC mechanical properties. Overall, these low throughput methods inevitably perturb the samples and provide poor statistics on the population level. In the past few years, quantitative phase microscopy (QPM) (45) has also been used to study biomechanics of sickle cell disease at single cell level (46, 47). Nonetheless, this approach has so far been used only under fully oxygenated conditions. Development of new non-contact optical assays is needed to study the biomechanics of sickle RBCs during hypoxia as well as to quantify the accurate mechansims / efficacy of existing FDA-approved as well as new drugs in the pipeline.

Approach: Three components of the TRD 2 can porivde quantitive tools for SCD investigation. High throughput tomographic microscope (TRD 2.2) will be used, for instance, to study fast shape changes in the individual RBCs during hypoxia via three-dimentional refractive index (RI) mapping. Interferometric 3D-resolved biomechanical assays (TRD 2.1) will be used to quantify biomechanical properties at normaxia and hypoxic conditions when the cytosol refractive index may be heterogeneous. Both of these technologies upon further developments can be used in more complex microfluidic environment such as ones with endothelial vasculature. Common-path interferometers with molecular-specific capability (TRD 2.3) promises to greatly increase the precision of the biomehcnical measurements at normaxic and hypoxic conditions. The technology developed in TRD2.3 will also provide additional valuable information on the concentration of the various proteins such as oxy / deoxy hemoglobin in the cytosol together with the morphological measurements of individual cells during the sickling process.

Push-Pull Relationship: The LBRC will **push** the development of the depth-resolved interferometric biomechanical assays to study the biophysics of sickle cell disease at the cellular level especially in low-oxygen enviroments (TRD 2.1). Trial of new drugs in Dr. Kato's group, such as anti-sickling agents, **pulls** all of the TRD 2.1, 2.2, and 2.3 that can provide quantitative tools to assess the efficacy of new treatments. These biomechanical studies can further lead to introduction of novel biophysical markers that can be associated with clinical outcomes and guide the care of patient on existing FDA-approved drugs (47). The need to extract rheological parameters from membrane fluctuations of sickle red cells in hypoxic state when cytosol mechanical property is heterogeneous further **pulls** LBRC to collaborate with Dr. Dao on developing finite element biomechanical models. Finally, initial discussions have been initiated with Dr. Tromberg in **pushing** this set of technology to study patient with sickle trait where their RBCs contain both HbA and HbS.

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