



Published in final edited form as:

*J Biomech.* 2017 January 04; 50: 34–41. doi:10.1016/j.jbiomech.2016.11.022.

## Biomechanics and biorheology of red blood cells in sickle cell anemia

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### Abstract

Sickle cell anemia (SCA) is an inherited blood disorder that causes painful crises due to vaso-occlusion of small blood vessels. The primary cause of the clinical phenotype of SCA is the intracellular polymerization of sickle hemoglobin resulting in sickling of red blood cells (RBCs) in deoxygenated conditions. In this review, we discuss the biomechanical and biorheological characteristics of sickle RBCs and sickle blood as well as their implications toward a better understanding of the pathophysiology and pathogenesis of SCA. Additionally, we highlight the adhesive heterogeneity of RBCs in SCA and their specific contribution to vaso-occlusive crisis.

### Keywords

Sickle cell disease; Erythrocyte; Biomechanics; Biorheology; Adhesion

## 1. Introduction

Sickle cell anemia (SCA), the first identified “molecular disease” (Pauling et al., 1949; Strasser, 1999), is one of the most common genetic inherited hematological disorders, which can cause several types of chronic complications such as vaso-occlusive crisis (VOC), hemolytic anemia, and sequestration crisis (Bunn, 1997; Barabino et al., 2010). The pathogenesis of vaso-occlusion involves several processes across multiple time and length scales, from  $\mathcal{O}(10^{-1}\text{s})$  to  $\mathcal{O}(10^3\text{s})$  for the kinetics of HbS polymerization to the hemodynamics of sickle blood flow, and from  $\mathcal{O}(10^{-9}\text{m})$  to  $\mathcal{O}(10^{-5}\text{m})$  for the size of the protein to the dimensions of the microcirculatory vessels. During the past few decades, different aspects of this disease have been successfully investigated (Barabino et al., 2010; Steinberg, 1999; Frenette and Atweh, 2007; Yazdani et al., 2016). At the molecular scale,

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**Conflict of interest statement**

None declared.

the HbS polymerization process has been characterized by a double nucleation mechanism. At the cellular scale, sickle RBCs are characterized by remarkable heterogeneity in density, morphology, and rigidity. The affected RBCs become more rigid and “sticky” compared to normal RBCs, causing frequent vaso-occlusive episodes and depriving tissues and organs of oxygen. At the microvascular scale, early studies postulated that the HbS polymerization resulted in the entrapment of sickle RBCs in capillaries (Fig. 1) (Kaul et al., 1986), and subsequent studies further revealed the *multi-interactional* and *multi-stage* nature of the VOC (Kaul et al., 1989, 1994, 2009; Kaul and Fabry, 2004).

Currently, hydroxyurea (HU) is the only approved medication in widespread use for the treatment of SCA (Ware, 2010). The treatment of SCA patients with HU has the following beneficial effects: (i) increased production of fetal hemoglobin (HbF) and therefore increased *delay time* of the RBC sickling process (Bridges et al., 1996; Atweh and Schechter, 2001), (ii) reduction of white blood cell (WBC) count and expression pattern of cellular adhesion molecules (Charache et al., 1996), and (iii) reduction in the frequencies of blood transfusion (Ware et al., 1999). These beneficial effects ameliorate the severity of SCA. However, clinical studies report that HU is ineffective for many patients for unclear reasons (Manwani and Frenette, 2013). Moreover, the aforementioned studies indicate that the clinical expression of SCA is heterogeneous, making it hard to predict the risk of VOC, resulting in a serious challenge for disease management. Here, we review experimental studies and predictive simulations related to biomechanical and biorheological properties as well as heterogeneity-related issues associated with SCA.

## 2. Biomechanical and biorheological properties of sickle RBCs

Quantification of the biomechanical and biorheological characteristics of RBCs can improve our understanding of the etiology of a number of human diseases. In SCA, partial deoxygenation of sickle RBCs in post-capillary venules causes HbS polymerization followed by possible RBC sickling. Repeated RBC sickling can result in the development of defects in the RBC membrane, reduced RBC deformability, increased time of RBC adherence to venules, and eventually in vaso-occlusion.

### 2.1. Sick cell biomechanics

Over the past few decades researchers investigated the biomechanics of sickle RBCs as indicators of the severity of the disease. The available experimental methods can measure the biomechanical properties of a large number of sickle RBCs at the same time (Chien et al., 1970; Messmann et al., 1990; Connes et al., 2014), or isolated sickle RBCs (Byun et al., 2012; Maciaszek and Lykotrafitis, 2011). For example, early studies using filtration (Chien et al., 1970) or ektacytometry (Messmann et al., 1990) directly examined the biomechanical properties of the sickle RBC membrane and determined that sickle RBCs are less deformable than normal RBCs. In a recent study, decreased RBC deformability and aggregation, measured using ektacytometry and laser backscatter of Percoll-separated sickle RBCs, have been shown to correlate with hemolysis (Connes et al., 2014). However, these techniques measure properties averaged over all RBCs in a blood sample, without regard to the cell heterogeneity within sickle blood sample. Single-cell experimental methods include

micropipette aspiration, optical tweezers, flickering analysis, atomic force microscopy (AFM), diffraction phase microscopy, and recently, microfluidics and ultrasounds. The optical tweezers and micropipette aspiration techniques subject the RBC directly to mechanical deformation and yield shear modulus of sickle RBCs in the range of 8–20  $\mu\text{N m}^{-1}$  (Fig. 2) (Byun et al., 2012). AFM measurements have found that the Young's modulus of SCA RBCs are stiffer than normal RBCs with a widely distributed Young's modulus ranged from 3 kPa to 50 kPa depending on the hypoxic conditions and probably on the clinical severity of the disease (Maciaszek and Lykotrafitis, 2011). The stiffening of sickle RBC membrane may indicate the effect of the polymerization of HbS as well as the possible remodeling of cytoskeleton associated with SCA.

Advances in mathematical models and computational simulations enable investigation of a broad range of biomechanical problems associated with RBCs in SCA. For example, Dong et al. (1992) developed a mathematical model of RBC flowing in narrow vessels. They showed that the RBCs become stiffer when the amount of intracellular HbS polymer increases. Hemolysis is associated with irreversible structural change of sickle RBCs (Kato et al., 2013). Therefore, the individuals with a higher numbers of irreversibly sickled cells (ISCs) are at greater risk for hemolysis (Serjeant et al., 1969). Fisseha and Katiyar (2012) employed a generalized Voigt-model of nonlinear viscoelastic solids to characterize the viscoelastic properties of sickle RBCs. They found that the ISCs with irreversible alteration in cell membrane structure tend to hemolysis.

## 2.2. Sick cell biorheology

Sickle RBCs have increased cell rigidity and decreased cell deformability, causing hemolysis and abnormal hemorheology in SCA (Chien et al., 1970; Usami et al., 1975). The rheological abnormalities are caused primarily by an increase in cytoplasm viscosity due to HbS polymerization upon deoxygenation (DeOxy), as well as biochemical abnormalities in the sickle RBC membrane. The abnormal rheological changes is also related to the cell membrane stiffening due to repeated cycles of polymerization and depolymerization in the circulating RBCs.

Sickle RBCs are heterogeneous in their rheological characteristics. According to Kaul et al. (1983), sickle blood is fractionated into 4 density groups (fractions I–IV). Fractions I (SS1) and II (SS2) are composed primarily of reticulocytes and discocytes, respectively, with mean corpuscular hemoglobin concentration (MCHC) levels similar to healthy RBCs, resulting in a comparable bulk viscosity to that of unseparated healthy blood samples in an oxygenated state (Kaul et al., 1983). Fractions III (SS3) and IV (SS4) are mainly composed of rigid discocytes and ISCs, respectively, with MCHC values considerably higher than those of healthy RBCs, which results in a significant increase in blood viscosity, even under oxygenated state (Kaul et al., 1983).

Recent experimental studies have captured information among all of the aforementioned processes in physiologic regimes, providing insight into the overall dynamics of a vaso-occlusive event (Du et al., 2015; Higgins et al., 2007; Wood et al., 2012; Lu et al., 2016b). In these experiments, whole blood or RBC suspensions from SCA patients flowed through microfluidic channels under constant pressure and low the oxygen concentration, thereby

simulating most basic features of a vaso-occlusive event. For example, Higgins and his colleagues found that oxygen tension regulate blood flow for individual SCA patients (Higgins et al., 2007; Wood et al., 2012; Lu et al., 2016b). Recently, Du et al. (2015) developed a high-throughput microfluidics-based model to investigate the sickle cell behavior under transient hypoxia (Fig. 3). Using this microfluidic device to measure blood samples from 25 SCA patients, they quantify the kinetics of cell sickling, unsickling, and individual cell rheology associated with SCA.

A change in blood rheological properties is usually linked to hematological diseases; therefore, the viscosity of blood has long been used as an indicator for understanding the implications and treatment routes of these type of diseases. Although various experiments have been performed for blood viscosity measurement in SCA, considerable uncertainty exists with respect to the effect of HU treatment on the viscosity of sickle blood: it has been reported that the viscosity may increase (Fattori et al., 2005), decrease (Ware, 2010) or even remain unchanged (Lemonne et al., 2015) for patients with SCA after treatment with HU.

Computational methods have been used to evaluate biorheological properties, to help explain the complex and multifactorial nature of SCA pathogenesis. For example, Dupin et al. (2008) employed a lattice Boltzmann method to simulate the sickle RBC suspension flowing through an aperture of diameter slightly less than the size of a single RBC. They found that the abnormally shaped and less deformable sickle RBCs get stuck upstream of the constriction. In recent years, a particle-based multiscale RBC (MS-RBC) model has been developed to simulate RBCs in disease like SCA (Lei and Karniadakis, 2013; Li et al., 2014, 2016; Chang et al., 2016). In this MS-RBC model, the cell membrane is represented by a triangulated network in two dimensions, which are connected by  $N_s$  edges and  $N_t$  triangles (Li et al., 2005; Pivkin and Karniadakis, 2008; Fedosov et al., 2010). The potential energy of the system includes in-plane elastic energy  $V_s$ , bending energy  $V_b$ , along with energies,  $V_{a+v}$ , from surface area and volume conservation. These energies are represented by

$$V_s = \sum_{j \in 1 \dots N_s} \left[ \frac{k_B T l_m (3x_j^2 - 2x_j^3)}{4p(1 - x_j)} + \frac{k_p}{(n-1)l_j^{n-1}} \right], \quad (1)$$

$$V_b = \sum_{j \in 1 \dots N_s} k_b [1 - \cos(\theta_j - \theta_0)], \quad (2)$$

$$V_{a+v} = \sum_{j \in 1 \dots N_t} \frac{k_l (A_j - A_0)^2}{2A_0} + \frac{k_v (V^{tot} - V_0^{tot})^2}{2V_0^{tot}}, \quad (3)$$

where  $k_b$  is the bending coefficient,  $l_j$  and  $l_m$  are the stretched length and contour length of spring  $j$ ,  $\theta_j$  and  $\theta_0$  are the instantaneous angle and spontaneous angle between two adjacent triangles. Also,  $A_j$  is the instantaneous triangle area, and  $A_0$  is the initial triangle area.  $V^{tot}$  is

the total RBC volume, while  $V_0^{tot}$  is the initial total RBC volume. The MS-RBC model represents seamlessly the healthy and infected RBCs as well as the plasma flow, therefore, it has been widely used to study the behavior of normal and abnormal RBCs in microfluidics. For example, Lei and Karniadakis (2012b) quantified the hemodynamics of sickle RBC suspensions under various physiological conditions. They showed that the deoxygenated sickle RBCs exhibit “solid” behavior and the viscosity of deoxygenated sickle RBC suspension is nearly shear-independent throughout the entire shear rate regime. Li et al. (2016) examined the effect of the cell morphology on the rheology of sickle RBC suspension under shear. They showed that the abnormal rheological properties of sickle RBCs are correlated with the cell morphology (Fig. 4).

It is known that the origin of SCA can be traced to a common molecular basis, but individual patients with SCA have a highly variable clinical phenotype. For these reasons, Li et al. (2016) have recently developed a predictive *patient-specific* model of SCA at the molecular level to quantify the rheological behavior of blood flow in SCA. They determined the shear viscosity of blood from SCA patients with HU treatment and those without HU treatment. Their results demonstrated that treatment with HU can indeed improve the blood flow in SCA.

### 3. Adhesive properties of sickle RBCs

As noted above, cell sickling is necessary but not sufficient to initiate a VOC. Abnormal adhesive properties of sickle RBCs, including activation of known adhesion receptors and increased interactions with WBCs, platelets, ECs, and extracellular matrix proteins, have drawn intense attention as potential initiating factors in VOC (Hillery et al., 1996; Hebbel et al., 2004). Here we overview significant studies and contributions to this field.

#### 3.1. Adhesive dynamics

Increased adhesive forces between sickle RBCs and ECs have been hypothesized to play a role in the initiation of vaso-occlusion in SCA. *In vitro* studies have shown that sickle RBCs exhibit heterogeneous cell adhesivity among different density groups (Barabino et al., 2010, 1987). The dense ISCs were found to preferentially adhere to ECs in static assays (Wautier et al., 1985), but other researchers demonstrated that the light-density RBCs adhere more avidly to ECs than ISC-rich dense group under flow conditions mimicking those in postcapillary venules (Barabino et al., 1987). It is likely that the irregular geometry and decreased deformability of dense ISCs prevent the type of contact needed to promote adhesion under shear forces induced by fluid flow. In a recent study, Alapan et al. (2014) presented a microfluidic approach to study the abnormal adhesive properties of individual sickle RBCs in physiological flow conditions. They demonstrated that the most tightly adherent cells are non-deformable sickle RBCs as they exhibit increased adhesion sites compared to the deformable ones.

The adhesion of sickle RBCs is also protein-dependent (Lee et al., 1998; Joneckis et al., 1996). Previous studies have shown that the laminin receptor on sickle RBCs is more available in denser groups (Lee et al., 1998), while thrombospondin supports higher levels of adhesion of light-density sickle RBCs (Joneckis et al., 1996). Epinephrine is a hormone that

is increased in the midst of stress. It has been shown that adhesion of sickle RBCs to the endothelium increases in the presence of epinephrine (Telen, 2005; Hines et al., 2003; Zennadi et al., 2004, 2007; Maciaszek et al., 2014). Epinephrine stimulates G-protein coupled receptors which activate the cyclic AMP-protein kinase A (cAMP-PKA) controlled pathway through stimulation of adenylate cyclase (AC) which in turn activates RBC surface adhesion receptors such as BCAM/Lu and ICAM-4 (Hines et al., 2003; Zennadi et al., 2004). Maciaszek et al. (2014) demonstrated that activation of these adhesion receptors is mediated by the scaffolding A-kinase anchoring proteins (AKAPs).

Some studies also probe the effect of HU therapy on the adhesive properties of sickle RBCs (Hillery et al., 2000; Gambero et al., 2007). Therapy with HU has been shown to decrease sickle RBC adhesion and downregulates endothelial adhesion molecules such as thrombospondin, laminin, and fibronectin. For example, Gambero et al. (2007) used a static adhesion assay to analyze the basal adhesion of RBCs, from SCA patients with HU treatment and those without HU treatment, to fibronectin. They found that HU therapy is associated with reduced adhesion molecule gene and protein expression in sickle RBCs with a concomitant reduction in adhesive properties. Odièvre et al. (2008) suggest that HU may modulate the activation of adhesion receptors by directly acting on gene expression and the related signaling cascade. Maciaszek et al. (2014) investigated RBC–EC interactions and their regulation via cAMP using single-molecule AFM experiments. They showed that HU treatment results in a reduced adhesion between sickle RBCs and ECs due to lower receptor expression. Hence, overall, the HU therapy reduce the complications of SCA.

Computational models have been employed to quantify the adhesive properties of sickle RBCs (Lei and Karniadakis, 2012b, 2013). For example, Lei and Karniadakis (2013) examined the adhesive dynamics of sickle RBCs of different density groups. Given the same “adhesive potential”, their results validate the hypothesis that heterogeneous cell adhesive dynamics is mainly due to the different cell rigidities and peculiar cell morphologies (Kaul et al., 1994). A deformable SS2 cell exhibits firm adhesion to the surface with a large contact area, a rigid SS3 cell shows weak adhesivity, while an ISC does not show any adhesion to the surface; instead, it moves freely without adhesive bonds established thereafter. In addition, they computed the adhesive force between the surface and sickle RBCs. They found that the adhesive force exhibits an inverse relationship with the cell rigidity: compared with the discocyte, the ISC exhibits smaller adhesive force given the similar cell rigidity, indicating less adhesivity induced by its peculiar cell morphology.

### 3.2. Vaso-occlusive crisis

VOC is the hallmark of the biophysical characteristics related to SCA. Typically, it has been viewed as synonymous with the blockage of single sickle/elongated RBC in capillaries (Kaul et al., 1986). Subsequent studies revealed that VOC is a multistep and multicellular paradigm (Kaul et al., 1989, 1994; Kaul and Fabry, 2004). Several models of vaso-occlusion have been proposed. For example, in a two-step model proposed by Kaul and his colleagues, preferential adhesion of deformable sickle RBCs in post-capillary venules that is followed by selective trapping of dense sickled RBCs could result in vaso-occlusion; alternatively, WBC adhesion in inflamed venules could trigger the selective trapping (Kaul and Fabry,



2004). An alternate model is proposed by Frenette (2002), in which sickle cell vaso-occlusion is viewed as a multistep and multicellular process driven by inflammatory stimuli and the vaso-occlusion is mediated by a number of cellular elements in four steps: (i) endothelial activation, (ii) WBC adherence to endothelium, (iii) sickle RBC interactions with adherent WBCs, and (iv) progressive blockage.

Computational modeling and simulations have also been used to tackle the *multi-stage* and *multi-interactive* nature of VOC. For example, Lei and Karniadakis (2013) employed a multiscale model of sickle RBC to investigate the biophysical characteristics of the vaso-occlusion in SCA (Fig. 5). They quantified the specific physiological conditions triggering the vaso-occlusion crisis and identified the specific contribution of individual cell groups within the vaso-occlusion process. They found that under physiological conditions similar to microcirculation in post-capillaries, the interplay of deformable SS2 cells and ISCs can potentially trigger full blood occlusion. To probe the WBC recruitment and its effect to blood vaso-occlusion, Lei and Karniadakis employed the multiscale model of sickle RBC and WBC to simulate the cell suspensions (mixed sickle RBCs and WBCs) in a microtube (Fig. 5b). Starting from the initial condition, they found that the WBC migrates toward to the tube wall with firm adhesion, leading to a decrease in blood flow. The adherent WBC further interacts and traps the sickle RBCs, resulting in full occlusion. For venular flow with larger diameter, multiple WBC recruitment may occur in the inflammation regions. They also simulated the sickle RBC suspension with multiple WBCs in a larger microtube. They found that the blood flow undergoes slow down due to the WBC recruitment and the moderate sickle RBC–WBC interaction leads to multiple sickle RBC trapped on the WBCs and the full occlusion (Fig. 5b).

## 4. Hemoglobin properties

SCA is a molecular disease that affects hemoglobin (Hb), the molecule in RBCs that delivers oxygen to cells throughout the human body. Many different types of Hb exist. The most common ones are normal HbA ( $\alpha_2\beta_2$ ), HbA<sub>2</sub> ( $\alpha_2\delta_2$ ), HbS ( $\alpha_2\beta_2^S$ ), HbC ( $\alpha_2\beta_2^C$ ), and HbF ( $\alpha_2\gamma_2$ ). Normal RBCs contain HbA composed of 2  $\alpha$ -globin subunits and two  $\beta$ -globin subunits; the pairing of one  $\alpha$  subunit and one  $\beta$  subunit produces a Hb dimer ( $\alpha_1\beta_1$ ). Two dimers combine to form a Hb tetramer ( $\alpha_2\beta_2$ ), the functional form of Hb. In SCA, the Glu-6 ( $\beta$ ) → Val mutation induces a hydrophobic interaction two neighboring HbS molecules (Bunn, 1997). HbS is less soluble than other forms of Hb under decreasing oxygen concentrations, leading to polymerization into long polymer fibers that deform the RBC shapes.

### 4.1. Sick cell hemoglobin in SCA

HbS nucleation followed by polymerization and RBC sickling significantly contributes to vaso-occlusion, which is the hallmark of SCA. The polymerization of HbS was modeled as a double nucleation process in Ferrone et al. (1985b) and Vekilov (2007). This nucleation is followed by the growth and alignment of HbS polymer fibers, transforming the cell into the classic sickle shape (Ferrone et al., 1985a,b; Samuel and Briehl, 1990; Vekilov, 2007; Mozzarelli et al., 1987). A different two-step mechanism of HbS polymerization has been

suggested by other groups (ten Wolde and Frenkel, 1997; Shiryayev and Gunton, 2004; Lutsko and Nicolis, 2006): (i) formation of dense liquid droplets, and (ii) formation of HbS fiber nuclei within the droplets. Consequently, HbS polymer fibers grow spontaneously and distort the RBCs into sickle shapes.

Once the Hb releases its oxygen, in SCA there is an ensuing molecular chain reaction between HbS molecules to form long polymer chains that are rigid. However, if the RBC is restrained inside a narrow capillary when this reaction begins, its shape is physically restrained from growing outward. In a recent study, Aprelev et al. (2012) employed a microfluidic method to investigate the mechanical interaction between sickled RBCs and capillaries. They constructed a single-cell microfluidic channel to identify the physical forces in RBCs and blood vessels underlying the painful symptoms of SCA. They found that the rigid elongated RBCs do not get stuck in narrow capillaries, and proposed that the timing of polymerization inside sickle RBCs may be important to understanding patients' susceptibility to symptoms.

Numerical models provide valuable insights into the mechanism of HbS fiber nucleation, polymerization and fiber growth. For example, Turner et al. (2003) proposed a theoretical model to study the thermodynamic stability of HbS of polymer fibers, demonstrating that twist plays an important essential role in stabilizing HbS polymer fibers. Yang et al. (2010) performed dynamic simulations of self-assembled filamentous bundles. They found that chain chirality can be used to control the HbS polymer bundle size. Li et al. (2012b) employed a coarse-grained HbS model to simulate the polymerization of HbS molecules. They demonstrated that the molecular chirality is the main driver for the HbS polymer fiber formation. Li and Lykotrafitis simulated the biomechanical properties of HbS polymer fibers via two different coarse-grained HbS fiber models, demonstrating the significant role of fiber frustration and compression in fiber zipping and unzipping dynamics (Li and Lykotrafitis, 2011; Li et al., 2012a). To gain insight into the nature of the formation of HbS polymer fiber, in a recent study, Lu et al. (2016a) developed a patchy particle HbS model to study the growth dynamics of HbS polymer fibers (Fig. 6a). They demonstrated that the formation process of HbS polymer fiber occurs through monomer addition. In addition, they found that the molecular chirality is the critical determinant of the mechanical and structural properties of HbS polymer fibers.

#### 4.2. Fetal hemoglobin in SCA

HbF is composed of 2  $\alpha$ -globin subunits and 2  $\gamma$ -globin subunits. Unlike HbA, HbF actively inhibits the polymerization of HbS, and as a result it reduces the severity of the disease. SCA patients with high HbF levels not only have less severe clinical course, but also show milder clinical complications, as increased production of HbF can reduce the occurrence of sickling-related complications (Akinsheye et al., 2011). Hydroxyurea promotes the production of HbF and can thus be used to treat SCA (Du et al., 2015; Charache et al., 1992; Ferster et al., 2001). In a mixed solution of HbF and HbS, there is a reduction of effective HbS concentration and, more importantly, both HbF and its mixed hybrid tetramer ( $\alpha_2\beta^S\gamma$ ) cannot enter the HbS polymer, making the RBCs less likely to sickle. In this way a small amount of HbF can reduce the ease with which sickling occurs, and thus ameliorate the



disease. In solution, HbF concentration higher than 15% prevents HbS polymerization (Bunn, 1987).

## 5. Summary and outlook

In this article, we review recent advances in probing and understanding the dynamics of collective processes associated with vaso-occlusion that links together sub-cellular, cellular, and vessel phenomena. We cover the biomechanical, biorheological and adhesive properties of sickle RBCs at cell level and abnormal hemoglobin properties at molecular level. The underlying molecular cause of the disease has been understood for more than half a century; however, progress in developing treatments to prevent painful VOC and the other myriad of associated symptoms has been slow (Parise and Berliner, 2016). Therefore, the need to develop new therapies or improve the existing treatments for SCA remains paramount. For these reasons, it is necessary to have a better understanding of the pathogenesis and pathobiomechanics of SCA and a more accurate evaluation of efficacy and safety of current anti-adhesive and deformability-restoring drugs in subsequent studies. Specifically, it is important to distinguish the effects of the current drug treatments, including HU, statins and adhesion-preventing drugs, from enhanced deformability and reduced adhesion, and examine what adhesive molecular mechanisms are influenced by other potential treatments, through which clinical inventions could potentially be designed and evaluated more effectively. This would potentially facilitate the design and evaluation of new clinical therapeutic approaches and interventions.

Aside from the intensive experimental studies, several computational approaches, including continuum- and particle-based methods, have been developed and applied to investigate a broad range of biomechanical and rheological problems associated with healthy and pathological RBCs at different length and time scales. Traditionally, continuum-based RBC models treat the RBC membrane and intracellular fluids as homogeneous materials, allowing the simulations of large-scale blood flow. However, they cannot describe the structural alteration of the RBC membrane in many hematological disorders such as the uncoupling of cytoskeleton from the lipid bilayer in SCA. In recent years, particle-based RBC models are increasingly popular as a promising tool for modeling of structural, biomechanical and biorheological properties of RBCs in disease. However, they are computationally expensive to scale up to large domains. From the perspective of computational modeling and simulations, the present computational framework can be further extended to investigate the following important questions related to SCA: (i) Development of a hybrid model that integrates the present particle-based RBC models at different levels (Fig. 6) with a continuum description of oxygen transport. Ultimately, computations that encompass all molecular and cellular scales could be used to investigate the entire process from deoxygenation to HbS fiber nucleation and then to vaso-occlusion in SCA. Such simulations would potentially answer questions concerning the links among HbS polymerization, cell sickling, blood flow alteration, and eventually VOC; (ii) Extension of the *patient-specific* model of SCA by including a macroscopic, continuum description of diffusion of chemicals (e.g., HU, decitabine, erythropoietin) to quantify the therapeutic effects of drug treatments on the microcirculation of the blood flow in SCA. These numerical studies, combined with

microfluidic experiments can be used to evaluate and quantify related therapeutic treatment and clinical outcomes.

## Acknowledgments

X.L., M.D., G.K. acknowledge support by the NIH grant U01HL114476. M.D. acknowledges partial support from the Singapore-MIT Alliance for Research and Technology (SMART) Center. G.L. acknowledges support by AHA grant 12SDG12050688, and by NSF grants CMMI-1235025 and PHY-1205910.

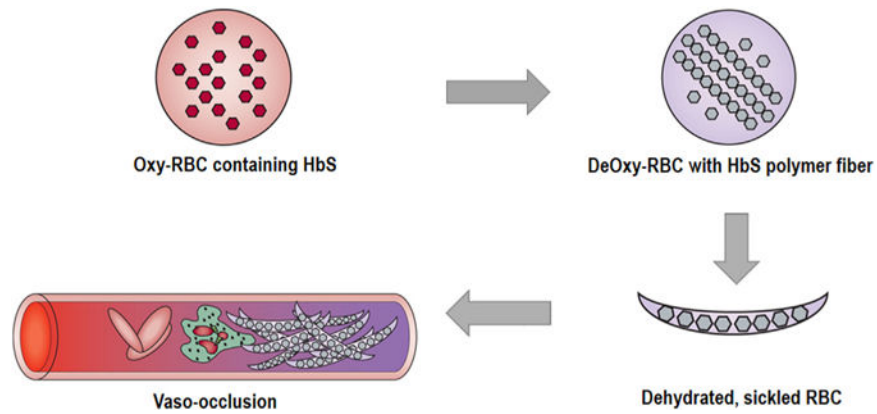
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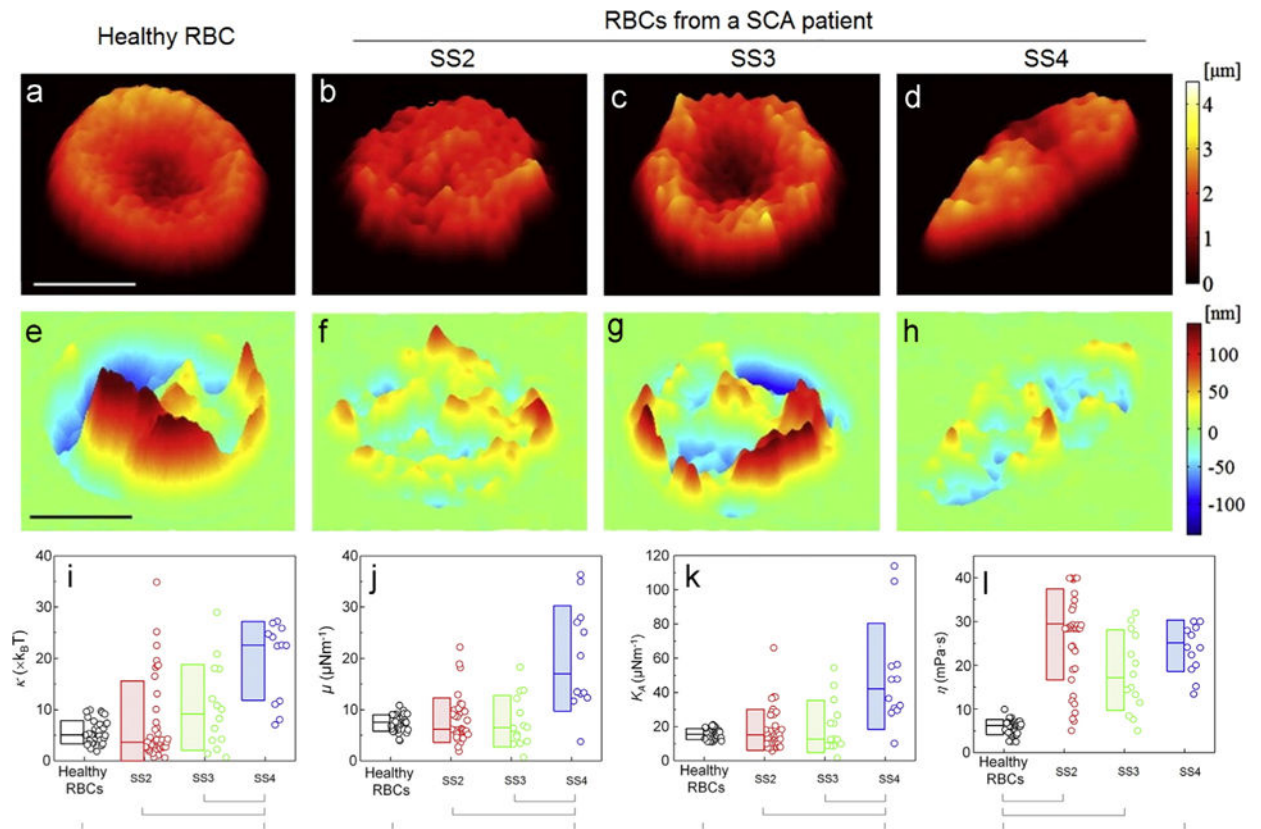
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**Fig. 1. Vaso-occlusive crisis in SCA**

Entrapment of sickle RBCs in microcapillaries. The polymerization of HbS molecules under Deoxy causes cell sickling and damage to the membrane. Some sickle RBCs get trapped in the microvasculature leading to vaso-occlusion. Adapted with permission from Rees et al. (2010).

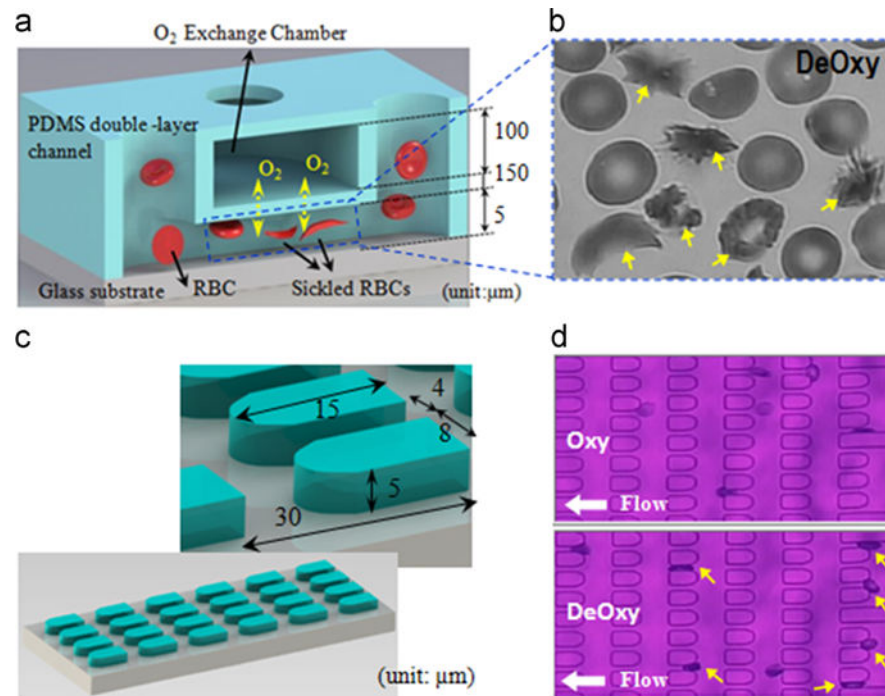




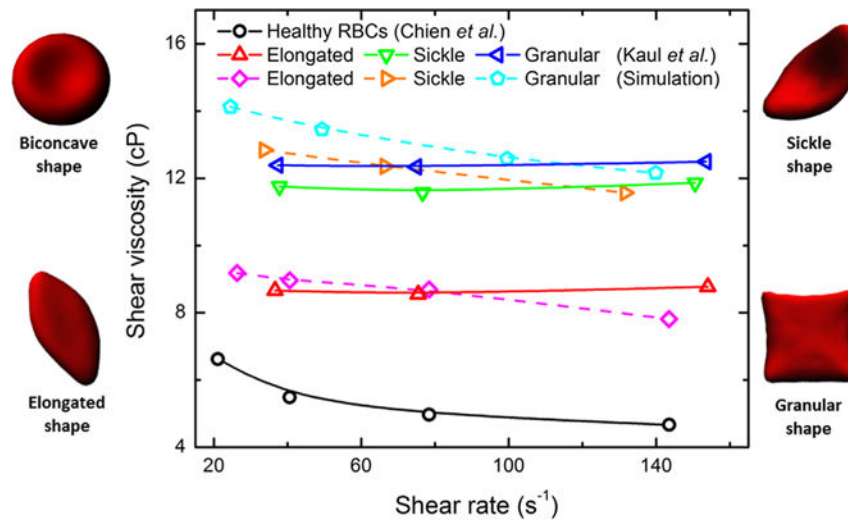
**Fig. 2. Biomechanical properties of RBCs in health and in SCA**

(a–d) Topographies of individual RBCs from healthy individuals (a), SS2 sickle RBC (b), SS3 sickle RBC (c), and SS4 sickle RBC (d). (e–h) Instantaneous membrane displacement maps of RBCs in (a–d). Measurements of (i) bending modulus  $\kappa$ , (j) shear modulus  $\mu$ , (k) area modulus  $K_A$ , and (l) viscosity  $\eta$  for healthy, and SS2, SS3 and SS4 RBCs in SCA.

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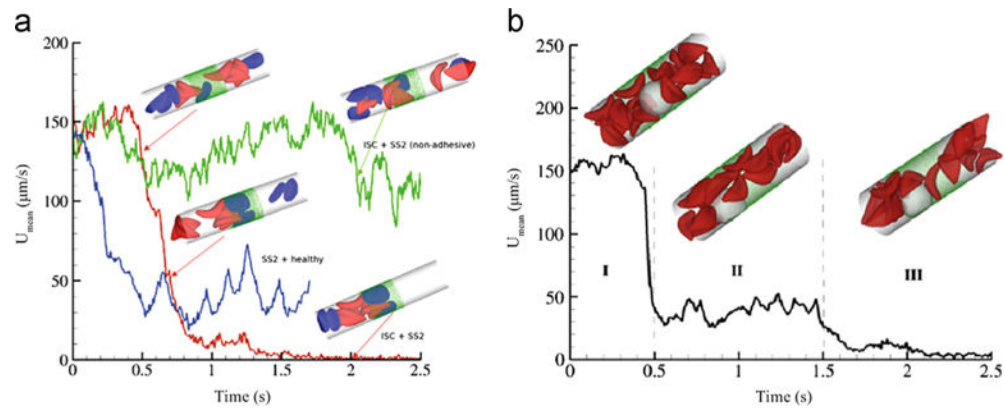


**Fig. 3. Microfluidic platform for studying sickle cell behavior under transient hypoxic conditions** (a) Schematic diagram of microfluidic device created for investigation of the kinetics of cell sickling and unsickling. (b) Visual determination of cell sickling events from morphological changes in cell membrane. (c) Schematic diagram of microfluidic device for studying single-cell rheology. The microfluidic channel, which contains periodic obstacles forming 15- $\mu$ m-long, 4- $\mu$ m-wide and 5- $\mu$ m-high microgates, mimics the capillaries. (d) Individual cell behavior under Oxy and DeOxy states. Yellow arrows indicate sickled RBCs. Reprinted with permission from Du et al. (2015).



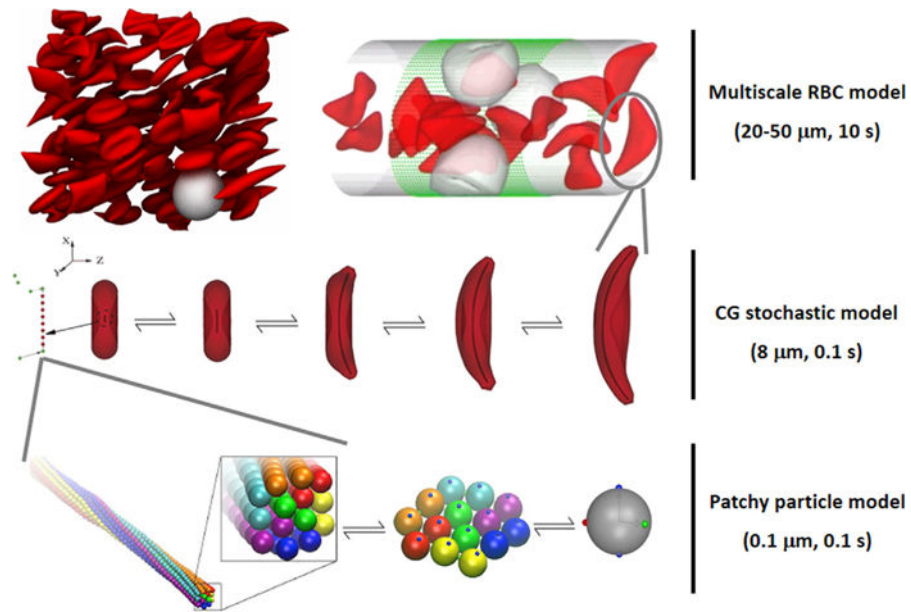
**Fig. 4. Shear viscosity of sickle cell suspension with different cell morphology at different shear rate**

For comparison, the experimental measured shear viscosity of whole blood in health at Hct=45% by Chien et al. (1966) and in SCA at Hct=40% by Kaul and Xue (1991) are shown in this figure. Reprinted with permission from Li et al. (2016).



**Fig. 5. Vaso-occlusion in post-capillaries**

(a) Instantaneous mean velocity of blood flow in a microtube of  $D=10 \mu m$  with different sickle RBC suspensions. The red, green and blue curves show the simulations results obtained from adherent-SS2/nonadherent-ISC cell groups, nonadherent-SS2/adherent-ISC cell groups, and SS2/healthy cell groups, respectively. (b) Effect of WBCs on blood vaso-occlusion: instantaneous mean velocity of the blood flow in a microtube of  $D=13.4 \mu m$ . The inset snapshots show blood cells in free motion, WBC adhesion and vaso-occlusion states. Reproduced from Lei and Karniadakis (2013) by permission. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)



**Fig. 6. Multiscale modeling of SCA**

(*Lower*) Modeling of the growth of HbS polymer fiber at the 100 nm length scale via coarse-grained patchy particle model. Adapted from Lu et al. (2016a) with permission from Elsevier. (*Middle*) Modeling of cell morphological sickling at the 8  $\mu\text{m}$  length scale via coarse-grained stochastic model. Reproduced from Lei and Karniadakis (2012a) with permission from Royal Society of Chemistry. (*Upper*) Modeling of sickle blood suspension in microchannel (*Left*) and microtube (*Right*) with their size up to 50  $\mu\text{m}$  via MS-RBC model. Reproduced with permission from Li et al. (2016) and Lei and Karniadakis (2013).