REVIEWS

This section of Journal of Materials Research is reserved for papers that are reviews of literature in a given area.

Mechanical response of human red blood cells in health and disease: Some structure-property-function relationships

S. Suresh[a)]

Department of Materials Science and Engineering, Division of Biological Engineering, and Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139-4307

(Received 28 March 2006; accepted 19 May 2006)

Aspects of mechanical deformability and biorheology of the human red blood cell are known to play a pivotal role in influencing organ function as well as states of overall health and disease. In this article, consequences of alterations to the membrane and cytoskeletal molecular structure of the human red blood cell are considered in the context of an infectious disease, *Plasmodium falciparum* malaria, and several hereditary hemolytic disorders: spherocytosis, elliptocytosis, and sickle cell anemia. In each of these cases, the effects of altered cell shape or molecular structure on cell elasticity, motility, and biorheology are examined. These examples are used to gain broad perspectives on the connections among cell and subcellular structure, properties, and disease at the intersections of engineering, biology, and medicine.

I. INTRODUCTION

As an area of scientific and technological pursuit, "Materials Science and Engineering" has traditionally encompassed broad intellectual activities that probe the connections among the processing, micro/nanostructure, properties, and performance of engineered materials. This sphere of activities has long involved such disciplines as solid state physics, chemistry, applied mathematics, and essentially all aspects of engineering and technology. In recent years, the rapid expansion in the scope of traditional subspecialties of materials science and engineering has led to greater incorporation of such themes as experimental and computational biology, biophysics, biochemistry, biomedical engineering, medicine, and genomics in the context of natural and synthetic biomaterials and their functions within the human body.

With advances in nanotechnology, the ability to probe the structural features and the size dependence of various properties of engineered and biological materials down to size scales finer than a nanometer has led to unprecedented opportunities for scientific investigations at the intersections of engineering, biology, and medicine. Concomitant with this development, recent advances in computer hardware and software have led to ever-improving sophistication in the computational modeling of the structure and properties at multiple length scales. These developments, along with substantial progress in bioimaging and genomics, have collectively provided new capabilities for the study of biological cells and molecules. A particular topic of expanding research interest in this broad area involves the mechanical responses of single biological cells and molecules and their connections to human diseases. Specifically, the common availability in recent years of biophysical tools to mechanically probe cells and molecules in fluid media at force and displacement resolutions of picoNewtons and nanometers, respectively, has facilitated quantitative and systematic experimental studies of how biochemical factors alter the cellular and subcellular mechanical properties that, in turn, influence, and are influenced by, disease states.

Investigations of the nanomechanics of living cells and subcellular molecular network structures also provide challenging new opportunities to probe the structure-properties-function paradigm intrinsic to the area of materials science and engineering in unique ways.[1–4] In this regard, as illustrated in Fig. 1, the following issues are of considerable interest for the study of human diseases:

(i) How do biological processes instigated by chemical effects occurring naturally in the body, by the external environment, by hereditary factors, or by invasion of a foreign organism into the human body lead to molecular changes in the cell surface, membrane, and cytoskeleton?

(ii) How do these structural changes lead to changes in the mechanical characteristics of the whole cell, in the manner in which the cell adheres to other cells, and in the

[a)] Address all correspondence to this author.
e-mail: ssuresh@mit.edu
DOI: 10.1557/JMR.2006.0260

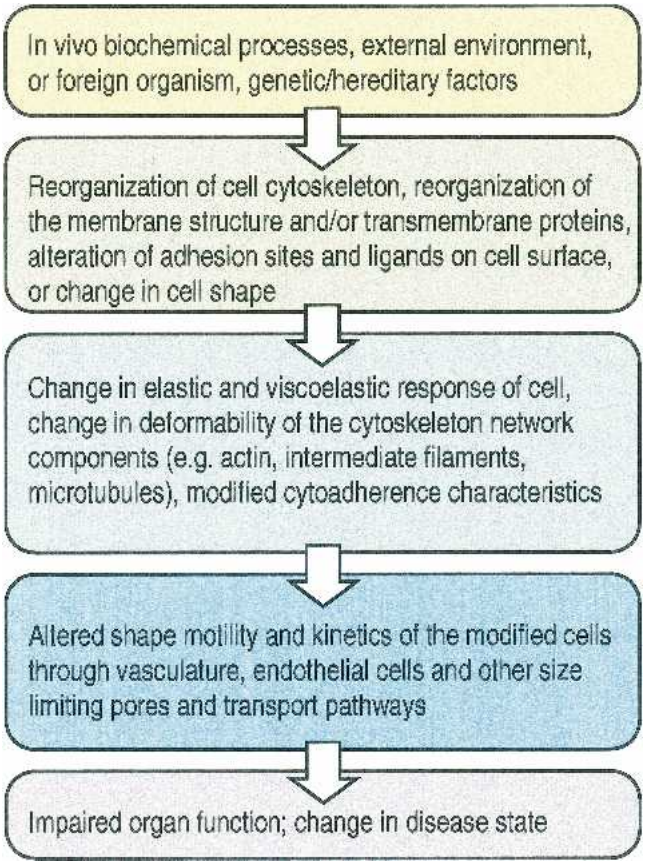

FIG. 1. Chemobiomechanical pathways in the connections among structure-properties and disease state of cells.

rheology and motility of the cell, intercellular regions, and pores?

(iii) How does the altered cell rheology and motility and cytoadherence influence health/disease?

In this article, we examine specific examples of the connections between the mechanical responses of living cells and specific human diseases in the context of an infectious disease and several hereditary diseases. For this purpose, in vitro studies of the mechanobiology of single cells and/or cell populations are examined for the following systems: human red blood cells (RBC) invaded by the malaria-inducing parasite *Plasmodium falciparum*, and human RBC with deficiency in cytoskeletal network of spectrin molecules as a consequence of hereditary hemolytic disorders such as spherocytosis, elliptocytosis, or Asian ovalocytosis.

The perspectives and overview presented here are necessarily brief and are intended to convey only broad concepts. Further details on the topics addressed here, including a comprehensive survey of recent advances and lists of key references, can be found in the papers cited in this brief review. For an introduction on the structure of the human RBC, the reader may find it useful to consult a textbook on cell biology.

II. INFECTIOUS DISEASE: MECHANICAL RESPONSE OF RBC INVADED BY *P. FALCIPARUM* MEROZOITES

The cause of malaria, the disease that affects nearly 8% of the world population, causing nearly 2 to 3 million deaths annually,[5] is the protoctistan parasite *Plasmodium*. Of the four species of *Plasmodium*, the most common are *P. falciparum* (which is responsible for most of the mortality) and *P. vivax*. Transmission of parasites between human hosts and mosquito vectors occurs when a female *Anopheles* mosquito feeds on human blood to acquire the necessary protein to make eggs. During feeding, millions of *P. falciparum* sporozoites residing inside the mosquito can be injected into a human along with the saliva of the mosquito containing an anticoagulant. The sporozoites travel in the human host to the liver through blood circulation, invade liver cells and multiply during the course of about 8 days. The liver releases millions of *P. falciparum* parasites in the form of merozoites (approximately 1 μm in diameter) into the blood stream. The merozoites penetrate RBC and continually change the structure of the RBC spectrin network (a two-dimensional brush-like structure tethered to the phospholipid bilayer that forms the cytoskeleton of the RBC) through interaction with the hemoglobin in the cytoplasm of the RBC and through transport of proteins to the spectrin network. A single merozoite inside the RBC can nucleate 20 new merozoites during a period of 48 h (so-called asexual development stage) after invasion. These structural changes ultimately cause RBC lysis (destruction) and the release of newly nucleated merozoites, each of which can invade other RBC.[6–8]

During asexual development, the structural changes occurring in the RBC are commonly characterized into three stages[8]: (i) the ring stage with ring-like features that appear at about 30 min after merozoite invasion of the RBC and last for 24 h; (ii) the trophozoite stage with irregular knobs or bulges on the merozoite surface that lasts from about 24–36 h after invasion; and (iii) the schizont stage in which the parasite undergoes nuclear division, spreads within the RBC, changes the RBC to a spherical shape, and makes further modifications to the RBC cytoskeleton and membrane during the period of 36–48 h after invasion.

The mechanical deformation characteristics of RBC infected with *P. falciparum* have traditionally been investigated through such techniques as micropipette aspiration and laminar shear flow (see Refs. 2 and 9–11 for a review of these methods). Recent advances in cell deformation techniques, such as optical tweezers,[12–15] have led to more controlled and direct uniaxial tests of the RBC, with a force resolution as small as 1 pN, at different developmental stages of the parasite during the asexual cycle. Figure 2 shows an example of large-deformation optical tweezers stretching a healthy RBC

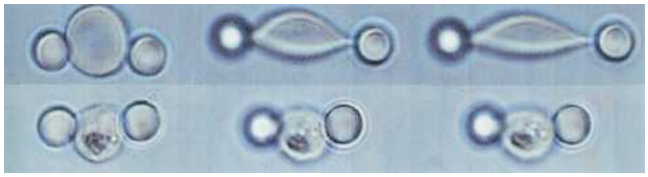

FIG. 2. Optical microscopy images of a healthy human RBC and RBC invaded by the malaria-inducing parasite *P. falciparum*. The cells are attached to glass beads at opposite ends and stretched by laser tweezers in a phosphate-buffered saline medium at room temperature. The top row shows a healthy RBC and the bottom row shows the cell with the parasite in it at 36 h postinvasion. Images show no force (left column), a force of approximately 70 pN (middle column), and a force of approximately 150 pN (right column). The parasite-invaded cell loses its ability to stretch. See Refs. 1 and 15 for details on the experimental method. Images adapted from Ref. 1.

and an infected RBC (schizont stage) in a phosphate-buffered saline solution at several different force levels.

The effects on the elastic response of the RBC as a function of the intracellular developmental stage of the merozoite are illustrated in Fig. 3. The results[1] indicate that during the course of a 48 h period after invasion of the RBC by the merozoite, the effective stiffness of the cell increases by more than a factor of 10. The effects seen from single-cell mechanical tests of reduced deformability on obstructed flow of invaded RBC have also been supplemented recently with RBC flow studies performed in microfluidic channels.[16,17] These experiments reveal that although healthy RBC (with a larger diameter of 7–8 μm) are able to pass through channels as small as 2×2 μm in cross section, schizont-stage infected RBC are easily obstructed at the entry point of channels of much larger cross sectional area.

The protrusions and knobs that form on the surface of the RBC during the late trophozoite and schizont stages serve as adhesion sites for the binding of the infected RBC to healthy RBC, other infected RBC, dendritic cells, and endothelial surfaces.[1,6–8] The significant reduction in deformability and the marked increase in the "stickiness" of the RBC result in obstructed flow of the RBC through the microvasculature and impaired clearance by the spleen,[18,19] as summarized in Fig. 4. (The human spleen, a nonvital organ about the size of the fist, is located in the upper left corner of the abdomen and produces lymphocytes for the destruction and recycling of old RBC.) These mechanical factors associated with cell deformability and cytoadherence are considered to be key mechanistic pathways in the pathogenic basis of *P. falciparum* malaria.[6]

III. HEREDITARY DISEASES AND THE MECHANICAL RESPONSE OF RBC

The foregoing example dealt with severely altered mechanical characteristics of the human RBC as a consequence of external environmental factors that could eventually lead to *P. falciparum* malaria. However, there are a number of hereditary hemolytic disorders that result from defective proteins that bind the RBC spectrin network to the phospholipids bilayer or from a point mutation in the polypeptide chain in the hemoglobin molecule in the RBC. We now consider an example of each of these two types of hereditary defects in the context of the connections among subcellular molecular structure, cell mechanical response, and disease state.

A. Hereditary spherocytosis (HS)

As noted earlier, the human RBC contains a brush-like cytoskeletal network connected to the phospholipid bilayer. The elements of this network are α- and β-spectrin molecules that comprise domains, each of which is 106 amino acids long; the domains form a chain connected by flexible links. These structures, collectively constituting a connected network of spectrin molecules, are schematically illustrated in Fig. 5(a). The α- and β-spectrin molecules form a polygonal structure with junctions established at actin nodes. Different types of protein (ankyrin, band 3, glycophorin, band 4.1, and protein 4.2) connect the spectrin cytoskeleton to the lipid bilayer membrane of the RBC,[20,21] as shown in Fig. 5(b).

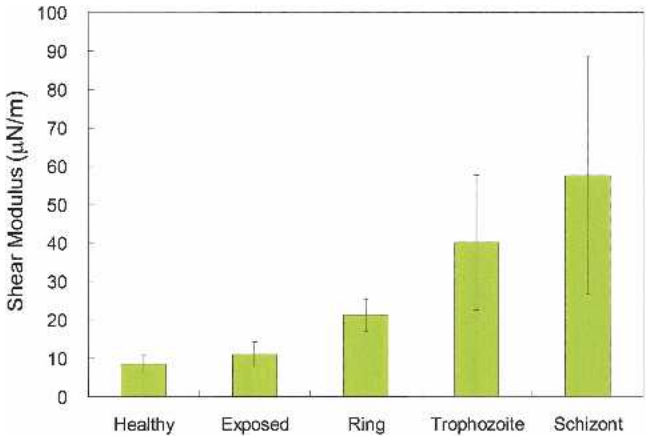

FIG. 3. Effective shear modulus of cell membrane as a function of the developmental stage of the *P. falciparum* merozoite (ring, trophozoite, and schizont) inside the RBC. This effective elastic response has been extracted from large-deformation optical tweezers experiments such as those shown in Fig. 2 and from three-dimensional computational simulations of optical tweezers stretching of RBC (see Refs. 1, 14, and 15 for procedures to extract moduli from optical tweezers experiments and computations). The control conditions shown include the healthy RBC and an "exposed" condition where the tested RBC does not host a parasite but is exposed in culture to other RBC harboring *P. falciparum* (whereby proteins exported by infected cells could potentially be transported to the outer surface of other cells). The stiffness of the exposed cell is slightly higher (based on repeat experiments involving at least five cells). The results shown are for room temperature. Data adapted from Ref. 1. The scatter bars represent the range of shear modulus values for each stage.

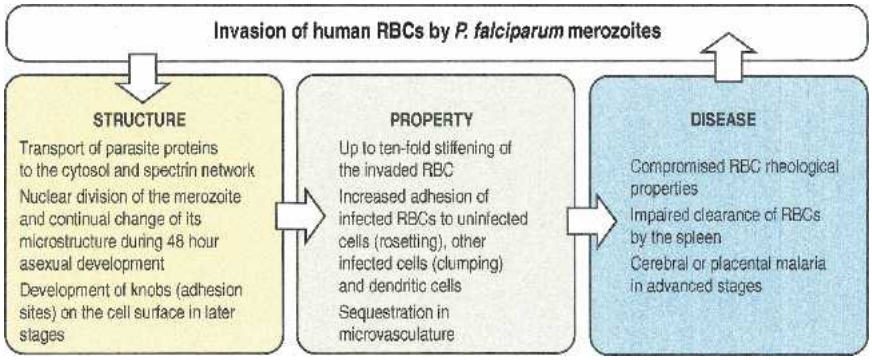

FIG. 4. Connections among structure, cellular, and subcellular mechanical properties and disease state in the case of human RBC invaded by *P. falciparum* merozoites.

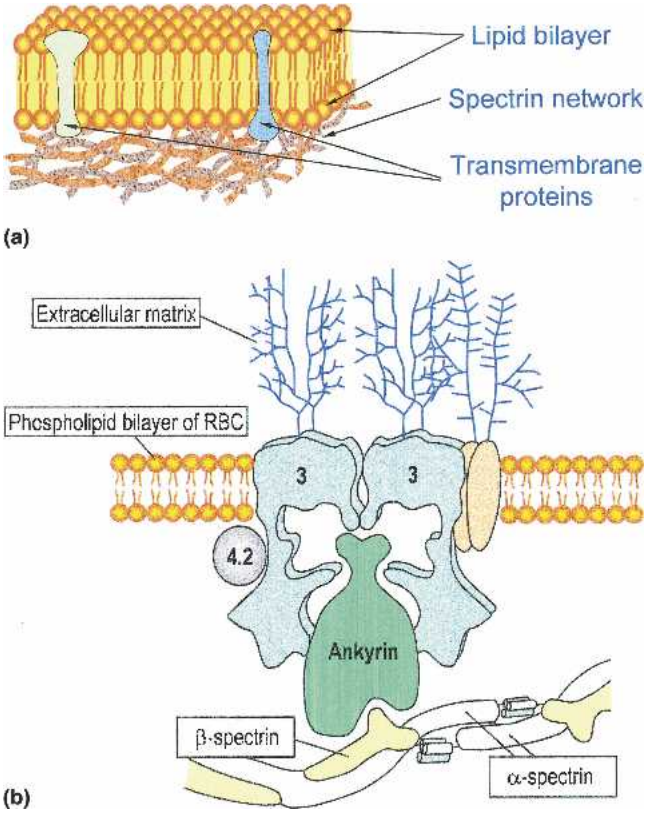

FIG. 5. Schematic illustration of the cytoskeletal structure of the human RBC and its attachment to the lipid bilayer. (a) Shows a predominantly two-dimensional brush-like network of α- and β-spectrin molecules forming the cytoskeleton of the RBC and attached to the phospholipid bilayer. (b) Shows Ankyrin, band 3, and protein 4.2 proteins that bind the RBC skeleton to the lipid bilayer. Adapted and modified from Refs. 20 and 21. Full details on the subcellular structure of the RBC can be found in Ref. 20.

HS is a blood cell disorder that arises from mutations of ankyrin and/or other membrane protein genes.[21,22] Although HS is found in all ethnic groups, it is most prevalent in northern Europe where 1 in 5000 people is susceptible. The RBC of an HS patient are characterized by a spherical shape of smaller diameter than the typical healthy RBC, which has a biconcave (discocyte) shape with excess surface area. The spherical RBC are less amenable than the healthy ones to traverse small blood vessels and microcapillaries, the smallest of which have an inner diameter of only 2.3 μm. The symptoms of the disease include an enlarged spleen (splenomegaly), jaundice, and anemia.[21]

In dominant HS, ankyrin, band 3, and β-spectrin exhibit mutations, whereas recessive HS occurs because of defective ankyrin, α-spectrin, or protein 4.2. The density of the spectrin network in the case of mild HS can be 80% of the spectrin density for a healthy RBC, and for severe HS, the spectrin concentration can drop to less than 50% of normal density.[21] Here, the density refers to the extent of connectivity or the number of connected nodes over the total number of nodes. (In other words, full density implies the absence of "holes" in the network). The disconnection of the spectrin skeleton from the lipid bilayer resulting from HS is accompanied by vesiculation of unsupported lipid bilayer. This "budding off" process leads to a gradual reduction in membrane surface area and to an eventual spherical shape where the bilayer and transmembrane cholesterol content is reduced by as much as 20% relative to that of a healthy RBC.

Although homozygosity for dominant HS is nearly fatal, splenectomy is one of the procedures performed for HS.[23] However, such therapy should be viewed with caution in that the spleen is an important organ for clearing foreign organisms, such as malaria parasites, from the body. Consequently, splenectomized patients are exposed to greater risk of succumbing to infectious diseases.

A strong correlation exists between the RBC mechanical biorheology and HS. The compromised deformability of RBC in HS patients is usually characterized by recourse to a "deformability index" (DI).[25] It provides a laboratory indication of the relative ease with which an RBC deforms during blood circulation in an HS patient as a function of the variations in the osmality of the suspending medium (measured in units of mOsmol/kg). Quantitatively, DI is a measure of the ellipticity of the

cell (i.e., it represents the ratio of the minor to the major axis of the cell), which is an indirect indictor of the extent to which the cell is able to change its shape and deform during microcirculation. The experimental method used to extract this information is known as osmotic gradient ektacytometry of the RBC using a laser diffraction viscometer.[25] This method provides a straightforward way to detect changes in RBC water content and surface area, and to infer the ability of the cell to traverse in blood circulation in microcirculation. Figure 6 shows a plot DI as a function of spectrin density changes arising from HS for a fixed osmolality of 300 mOsmol/kg.

B. Hereditary elliptocytosis (HE)

Other types of shape mutations of RBC resulting in compromised deformability arise from HE. Mutations in α-spectrin, β-spectrin, glycophorin C, or protein 4.1 genes are considered responsible for HE, which is most prevalent among people of Mediterranean and African ancestry, ostensibly because it provides some resistance to malaria.[26,27] HE, like HS, causes the cytoskeleton and membrane of the RBC to become less deformable. The effects of protein 4.1 deficiency on mechanical stability and deformability are illustrated in Fig. 7.

C. Sickle cell anemia

A healthy human RBC contains approximately 250 million hemoglobin molecules.[20] Four polypeptide chains, two of type α and two of type β, form the globular protein hemoglobin. In people with sickle cell disease, a mutation occurs in the starting sequence of the

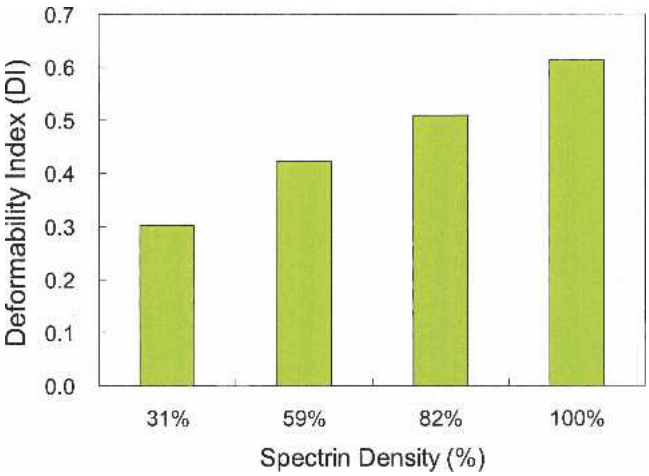

FIG. 6. Relative change in deformability of the spherical RBC as a function of spectrin concentration at a fixed osmolality of 300 mOsmol/kg. The greater the loss of RBC surface area, the lower is the value of DI. In more hypotonic solutions (lower osmolality), a decrease in surface-to-volume ratio causes a reduction in DI at a fixed spectrin density. In hypertonic solutions, cellular dehydration results in lower DI. After Walensky et al.[24]

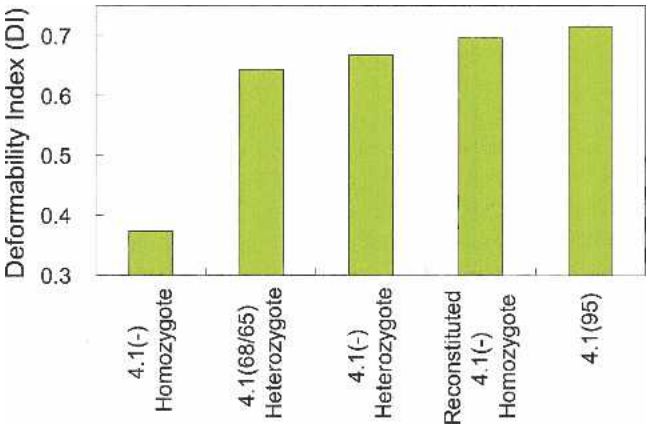

FIG. 7. Changes in DI from defects in protein 4.1 that cause HE. The results shown here are for RBC subjected to shear stress for 20 s in an ektacytometer. Cells completely devoid of protein 4.1 [homozygote 4.1(−1)] have the lowest DI; their deformability is restored by reconstituting with normal protein 4.1, DI values close to those of the normal range, 4.1(95). Heterozygote mutant cells, 4.1(68/65) and 4.1(−), have intermediate stability. Data adapted from Ref. 27.

amino acids in the β polypeptide chains. As a result, the normal sequence of

Val-His-Leu-Thr-Pro-Glu-Glu-Lys

is changed to

Val-His-Leu-Thr-Pro-Val-Glu-Lys

with the amino acid valine substituting for the amino acid glutamate in the sixth position of the hemoglobin β chain (hemoglobin S [HbS]).[28–30] This is a consequence of the fact that the allele for the gene coding for the β polypeptide in people with sickle cell anemia contains the base triplet CAT instead of the normal base triplet CTT. The portion of the polypeptide chain with this mutation lies on the surface of the hemoglobin where the water molecules present in the cytoplasm interact. Instead of the usual glutamate with its hydrophilic side chain, the variation containing valine with its hydrophobic side chain binds with another hydrophobic "dent" on the surface of another hemoglobin molecule whenever there is no oxygen present. Consequently, in the deoxygenated state (as, for example, when people with this disease perform strenuous exercise), the hemoglobin molecules combine to form a long fibrous shape, instead of the normal globular shape.[28] The RBC is then pulled into a "sickle" shape. The sickle-shaped RBC are not able to deform sufficiently to pass through small blood vessels. Thus, the altered shape leads to reduced deformability and obstruction of flow, causing a lack of oxygen supply.

Hemolytic anemia and vaso-occlusive crises that cause damage of the tissues can result in patients who are homozygous for the sickle cell mutation (HbSS). By contrast, people who are heterozygous for this mutation (HbAS or sickle cell trait) are asymptomatic.[30] One of

the common methods of treatments for the disease involves use of hydroxyurea (HU), which is known to promote RBC deformability and fetal hemoglobin production. Figure 8, derived from the work of Brandao et al.,[30] shows results from the optical tweezers measurements of elasticity of RBC from healthy patients, and those with the HbSS or HbAS mutation, as well as with the HbSS mutation but treated with HU for a period of at least 6 months. It is found that the RBC from homozygous and heterozygous patients were significantly stiffer than those of healthy subjects whose RBC served as the control. However, HbSS patients treated with HU had RBC whose elasticity was comparable with those of the healthy RBC. These results clearly illustrate how cell mechanics can serve to assess the existence of the disease as well as the efficacy of drugs used to treat the disease.

IV. CONCLUDING REMARKS

The examples illustrated in this review show the complex connections among the cellular/subcellular structure, cell mechanical response, motility, and disease state. In the case of *P. falciparum* malaria, the foreign organism invading the RBC leads to marked changes in its molecular structure, the consequences of which are increased rigidity, cytoadherence, compromised motility, and sequestration in microvasculature. In the case of hereditary hemolytic disorders such as spherocytosis, elliptocytosis, and sickle cell disease, the inherited mutations are found to markedly reduce the deformability of the RBC. Single-cell biomechanics assays are also shown to provide critical information about the existence of the disease, systematic alterations in cell structure, progression of disease state, and efficacy of drugs used to treat the disease.

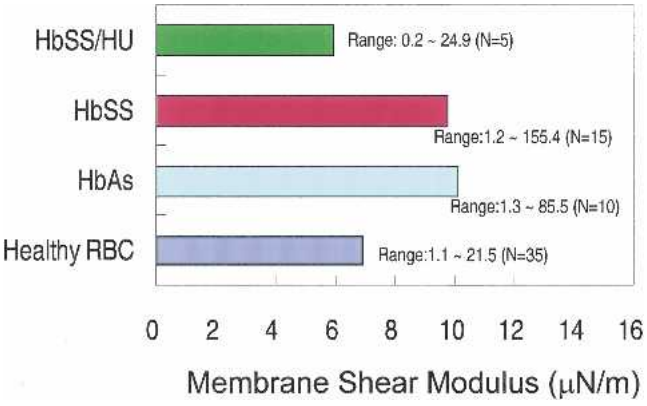

FIG. 8. Results of optical tweezers measurements at 25 °C of RBC at room temperature from healthy subjects and from patients homozygous (HbSS) and heterozygous (HbAS) for the sickle cell mutation. Also shown are the RBC elasticity results for HbSS patients treated for a minimum of 6 months with HU. The bar graph shows the median value of shear modulus. Also indicated are the range of values in units of μN/m. The number, N, in parenthesis indicates the number of tests performed for each case. (Data replotted from the results of Brandao et al.[30])

These examples point to the many complex interactions among cell biomechanics, deformability, shape evolution, biochemistry, genetics, and disease states. With the sophistication of available biophysical tools to probe the elastic and viscoelastic characteristics of cell membrane and subcellular components, it is now possible to gain a deeper understanding of the role of biomechanics in influencing disease states. These advances, in conjunction with the feasibility to clone organisms and to knock-out and/or knock-in specific proteins, have also provided new opportunities to probe the contributions of key proteins in the membrane and/or cytoskeletal structures of living cells to the nanomechanics of deformation. In addition, advances in micro- and nanofluidics offer exciting new possibilities to quantify the biorheology of cells in suspension and could some day provide a means to develop portable, disposable, and cost-effective diagnostic tools and drug efficacy assays that at least partly rely on advances in our understanding of chemomechanobiology. It is evident from the examples considered here that the structure-property-function connections traditionally sought in studies of the science and engineering of synthetic materials also offer a fertile ground for probing the mechanistic origins of human health.

ACKNOWLEDGMENTS

Preparation of this review was supported by the Advanced Materials for Micro and NanoSystems Program, as well as by the Computational Systems Biology Program of the Singapore-MIT Alliance. The author is very grateful to the members of his research group, especially to M. Dao, J.P. Mills, and M. Diez Silva, for many helpful discussions on several topics covered in this review.

REFERENCES

1. S. Suresh, J. Spatz, J.P. Mills, A. Micoulet, M. Dao, C.T. Lim, M. Beil, and T. Seufferlein: Single-cell biomechanics and human disease states: Gastrointestinal cancer and malaria. *Acta Biomater.* **1,** 16 (2005).
2. G. Bao and S. Suresh: Cell and molecular mechanics of biological materials. *Nat. Mater.* **2,** 715 (2003).
3. D.E. Ingber: Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ. Res.* **91,** 877 (2002).
4. D. Boal: *Mechanics of the Cell* (Cambridge University Press, Cambridge, UK, 2002).
5. B.M. Cooke, N. Mohandas, and R.L. Coppel: Malaria and the red blood cell membrane. *Semin. Hematol.* **41,** 173 (2004).
6. L.H. Miller, D.I. Baruch, K. Marsh, and O.K. Doumbo: Pathogenic basis of malaria. *Nature* **415,** 673 (2002).

7. B.M. Cooke, N. Mohandas, and R.L. Coppel: The malaria-infected red blood cell: Structural and functional changes. *Adv. Parasitol.* **50,** 1 (2001).
8. L.H. Bannister, J.M. Hopkins, R.E. Fowler, S. Krishna, and G.H. Mitchell: A brief illustrated guide to the ultrastructure of *Plasmodium falciparum* asexual blood stages. *Parasitol. Today* **16,** 427 (2000).
9. K.J. Van Vliet, G. Bao, and S. Suresh: The biomechanics toolbox: Experimental approaches for living cells and biomolecules. *Acta Mater.* **51,** 5881 (2003).
10. A. Evans: Bending elastic modulus of red blood cell membrane derived from buckling instability in micropipette aspiration tests. *Biophys. J.* **43,** 27 (1983).
11. F.K. Glenister, R.L. Coppel, A.F. Cowman, N. Mohandas, and B.M. Cooke: Contribution of parasite proteins to altered mechanical properties of malaria-infected red blood cells. *Blood* **99,** 1060 (2002).
12. A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, and S. Chu: Observation of single beam gradient force optical trap for dielectric particles. *Opt. Lett.* **11,** 288 (1986).
13. S. Henon, G. Lenormand, A. Richert, and F. Gallet: A new determination of the shear modulus of the human erythrocyte membrane using optical tweezers. *Biophys. J.* **76,** 1145 (1999).
14. M. Dao, C.T. Lim, and S. Suresh: Mechanics of the human red blood cell deformed by optical tweezers. *J. Mech. Phys. Solids* **51,** 2259 (2003).
15. J.P. Mills, L. Qie, M. Dao, C.T. Lim, and S. Suresh: Nonlinear elastic and viscoelastic deformation of the human red blood cell with optical tweezers. *Mech. Chem. Biosyst.* **1,** 169 (2004).
16. J.P. Shelby, J. White, K. Ganesan, P.K. Rathod, and D.T. Chiu: A microfluidic model for single-cell capillary obstruction by *Plasmodium falciparum*-infected erythrocytes. *Proc. Natl. Acad. Sci. USA* **100**, 14618 (2003).
17. C.T. Lim and S. Suresh: (unpublished research), National University of Singapore and Massachusetts Institute of Technology (2006).
18. R. Suwanarusk, B.M. Cooke, A.M. Dandorp, K. Silamut, J. Sttabongkot, and N.J. White: The deformability of red blood cells parasitized by *Plasmodium falciparum* and *P. vivax. J. Infect. Dis.* **189,** 190 (2004).
19. P. David and G. Milon: (private communication, Institut Pasteur, Paris, 2006).
20. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter: *Molecular Biology of the Cell,* 4th ed. (Garland, New York, 2002).
21. S. Eber and S. Lux: Hereditary spherocytosis: Defects in proteins that connect the membrane skeleton to the lipid bilayer. *Semin. Hematol.* **41,** 118 (2004).
22. C.F. Vanlair, J.B. Masius, and R. Bull: De la microcythemie. *Acad. Med. Belgium* **5,** 515 (1871).
23. S.W. Eber, A. Pekrun, and A. Neufeldt: Prevalence of increased osmotic fragility of erythrocytes in German blood donors: Screening using a modified glycerol lysis test. *Ann. Hematol.* **64,** 88 (1992).
24. L.D. Walensky: In *Blood: Principles and Practice of Hematology,* 2nd ed., edited by R.I. Handin, S.E. Lux and T.P. Stossel (Lippincott, Williams & Wilkins, Philadelphia, PA, 2003).
25. M.R. Clark, N. Mohandas, and S.B. Shohet: Osmotic gradient ektocytometry: Comprehensive characterization of red cell volume and surface maintenance. *Blood* **61,** 899 (1983).
26. P.G. Gallagher: Hereditary elliptocytosis: Spectrin and protein 4.1R. *Semin. Hematol.* **41,** 142 (2004).
27. N. Mohandas, M.R. Clark, and B.P. Health: A technique to detect reduced mechanical stability of red cell membranes: Relevance to elliptocytic disorders. *Blood* **59,** 768 (1982).
28. D.L. Nelson and M.M. Cox: *Principles of Biochemistry,* 2nd ed. (Garland, New York, 2005).
29. M. Jones and G. Jones: *Advanced Biology* (Cambridge University Press, Cambridge, UK, 1997).
30. M.M. Brandao, A. Fontes, M.L. Barjas-Castro, L.C. Barbosa, F.F. Costa, C.L. Cesar, and S.T.O. Sead: Optical tweezers for measuring red blood cell elasticity: Application to the study of drug response in sickle cell disease. *Eur. J. Haematol.* **70,** 207 (2003).