SICKLE CELL DISEASE (SCD) MANAGEMENT: A Theoretical Review

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ABSTRACT
We present in this study a theoretical review of Sickle Cell Disease (SCD), highlighting the history of the disease and its pathophysiology. In addition, we also spotlight the reactions involved in SCD, the kinetics and mechanism of hemoglobin oxygenation and deoxygenation in red blood cell (which is the bane for the change in cell geometry and membrane elasticity in sickle cell patients). Since SCD is a blood disease manifested in the polymerization and deformation of red blood cells, very small capillaries (≤ 10μm) are more prone to occlusion since irreversibly sickled cells (ISC) cannot pass through such vessels because of the loss in deformability. The mathematical models reviewed predicted occurrences (pertaining to red blood cell deformation and geometry) in the vessels to an appreciable accuracy but lacked the ability to predict vasoocclusion in the vessels.

Keyword: *SCD Management; Hemoglobin Oxygenation; Hemoglobin Deoxygenation; Polymerization of Red Blood Cells; Occlusion of Sickled Cells; Strenghts and Limitations of Theoretical Models*.

1. INTRODUCTION

Sickle cell disease (SCD) is a serious, hereditary and often life-threatening chronic disease (and quite common in Africa) in which the red blood cells have reduced life span and are rigid, with a crescent or sickle shape. The shape is the result of an abnormality in the hemoglobin, which alters the deformability of the cells under conditions of low oxygen tension. Because of their distorted shape, the cells have difficulty passing through the small arterioles and capillaries and have a tendency to clump together to start a 'logjam'. This occludes the blood vessels and obstructs blood flow and oxygen cannot reach the tissues on the other side of the logjam. This shortage of blood and oxygen is thought to be the genesis of the pains experienced in sickle cell disorder [1].

SCD is a blood disorder characterized by abnormal type of hemoglobin in the red blood cell called hemoglobin S (Hb S), which has less oxygen-carrying capacity. This molecular hemoglobin defect causes the polymerization of deoxygenated hemoglobins and results in reduced erythrocyte flexibility, deformation and numerous rheological effects. The red blood cells, RBCs, have a diameter of about 7μm. Due to their flexibility, the red blood cells are able to pass through capillaries of ≤ 3μm diameter. In the case of SCD, the red blood cells have lowered deformability due to repeated sickling (polymerization) and unsickling (melting) processes and eventually become irreversibly sickled and have to be removed from circulation. This happens frequently and accounts for the anemia characteristic of the disease. Occlusion of vessels is a common phenomenon as these sickled cells co-operate to form aggregates that later block the vessels.

SCD is a major health problem requiring lifelong multidisciplinary care to manage the wide range of medical and social consequences. Although a number of new approaches offer the potential to have an impact on the natural history of this disease, it is hoped that a permanent solution be found for the disorder in the nearest future.

Life expectancy of sickle cell patients has been found to increase with better management of the disease. For example, in 1973, in the USA, the average lifespan of a person with sickle cell anemia was 14 years. In 2003, it rose to 50 years. In African countries, despite the unavailability of statistics, the average lifespan of affected persons is also increasing as it is evident that more Nigerians with sickle cell anemia are living longer productive lives, beyond 50 years and a few have reached 60 – 80 years [1].

1.1 Pathogenesis of SCD

The hemoglobin molecule is made up of four polypeptide chains (globins): two alpha chains of 141 amino acid residues each and two beta chains of 146 amino acid residues each. The alpha and beta chains have different sequences of amino acids but fold up to form similar three-dimensional structures. Normal hemoglobin (Hb A) is formed by two type α and two type β polypeptide chains. In sickle cell hemoglobin (Hb S), the normal sequence of Valine-Histidine-Leucine-Threonine-Proline-Glutamic acid-Glutamic acid-Lysine is changed to Valine-Histidine-Leucine-Threonine-Proline-Valine-Glutamic acid-Lysine, with the amino acid valine substituting for the glutamic acid in the β6 (codon 6) site [2]. The gene defect is a known mutation of a single nucleotide of the β-globin gene and hemoglobin with this mutation is referred to as hemoglobin S (Hb S) as opposed to the more normal adult hemoglobin A (Hb A).

This imperfectly formed mutant forms an imperfect bond which limits its function as an oxygen-carrier. Hence, when there is a low concentration of oxygen, the hemoglobin S molecules polymerizes and can stick together in long, rigid chains inside the red blood cells. When this occurs, the red cells are forced to change from its usual disc-like shape to a banana or sickle shape. The red blood cells are then described as being sickled or to have undergone sickling. Sickling of the red blood cells may be temporary or permanent. When temporary, it is often reversed when the red blood cells are exposed to oxygen in the lungs but sooner or later, it becomes permanent and irreversible. Permanently sickled cells (also referred to as irreversible sickled cells, ISC) are rapidly removed from the circulation by the body's system for removing damaged cells.

Reduced cellular deformability of sickle red cells is primarily responsible for the increased viscosity of sickle blood. Clarke et al. [3] found that cellular dehydration and the consequent increase in cytoplasmic viscosity are major determinants of the abnormal rheological behavior of oxygenated sickle red cells. The deformation response of an individual red cell to applied force is a complex phenomenon that depends on a number of different cell characteristics, including membrane material properties, cell geometry and cytoplasmic viscosity [4].

In retrospect, some elements of the disease had been recognized earlier in Nigeria and known locally as *ogbanjes* ('children who come and go') because of the very high infant mortality in this condition. Also, the practice of using tar soap to cover blemishes caused by sickle cell sores was prevalent in the African American community.

1.2 Diagnosis of SCD

Attacks are diagnosed clinically, that is, there is no gold standard diagnostic test. Hemolysis (anemia and jaundice) is often present, although for painful crises the diagnosis depends essentially on how the patient describes the pain.

Abnormal hemoglobin forms are detected on hemoglobin electrophoresis, a form of gel electrophoresis on which the various types of hemoglobin move at varying speed. Sickle cell hemoglobin (Hb SS) and Hemoglobin C with sickling (Hb SC), the two most common forms can be identified from there. Genetic testing is rarely performed.

1.3Pathophysiology of SCD

New red blood cells are quite elastic, which allows the cells to deform to pass through capillaries. Repeated episodes of sickling causes loss of this elasticity and the cells fail to return to normal shape when oxygen concentration increases. These rigid red blood cells are unable to flow through narrow capillaries, causing vessel occlusion and ischemia.

Red blood cell deformability and its membrane properties are critical determinant of blood flow in the microcirculation. The life span of sickle cells is very much reduced to about $1/10\,\text{th}$ of normal cells (10 – 20 days) [5].

1.4 Blood: functions and components

The functions of blood include transporting oxygen and nutrients to the lungs and tissues, forming blood clots to prevent excess blood loss, carrying cells and antibodies that fight infection, bringing waste products to the kidneys and liver, which filter and clean the blood and regulating body temperature

Blood components are:

- **Plasma**: The liquid component of blood made up of a mixture of water, sugar, fat, protein, and salts and it helps to transport blood cells throughout the body along with nutrients, waste products, antibodies, clotting proteins… etc.
- **White Blood Cells (Leukocytes):** White blood cells protect the body from infection and account for about 1 percent of the human blood.
- **Platelets (Thrombocytes):** Platelets help the blood clotting process (or coagulation). **Red Blood Cells (Erythrocytes or RBCs):** Red cells are the most abundant cell in the blood, accounting for about 40-45 percent of its volume and known for their bright red color. The shape of a red blood cell is a biconcave disk with a flattened center.

1.5 Structure and composition of Red Blood Cells

The shape of erythrocytes is ideal for the function they perform. Seen from the top, erythrocytes appear to be circular but a side view shows that they are actually biconcave discs. This shape increases the surface area-to-volume ratio of the cell, thus increasing the efficiency of diffusion of oxygen and carbon dioxide into and out of the cell.

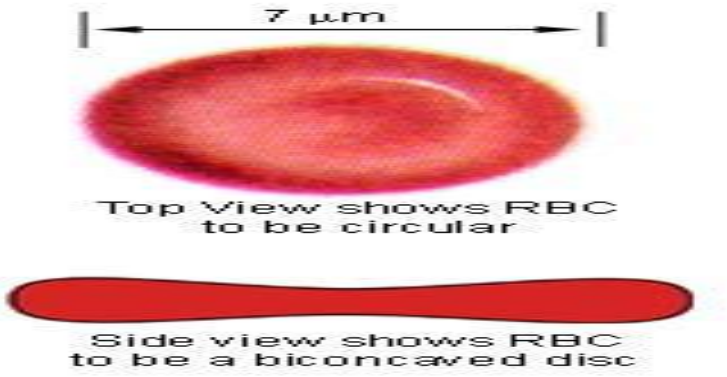


Fig. 1.1: Top and Side view of RBC [6]

Erythrocytes are biconcave disks having a flattened center as shown in Fig. 1.1 and also have a flexible plasma membrane. This feature allows erythrocytes, which have a 7µm diameter, to squeeze through capillaries as small as 3µm wide. Erythrocytes contain tremendous amounts of hemoglobin, the protein that binds oxygen.

Because they lack a nucleus and other cellular machinery, erythrocytes cannot repair themselves when damaged; consequently they have a limited life span of about 120 days. The removal of old and dying erythrocytes is carried out by the spleen. Erythrocytes die at a rapid rate of 2-3 million erythrocytes every second. Erythrocyte production must equal erythrocyte death or the cell population would decline. Erythrocytes are produced through a process called erythropoesis.

1.5.1 Hemoglobin

Red cells contain a special protein called hemoglobin. Blood appears red because of the large number of red blood cells, which get their color from the hemoglobin. The percentage of whole blood volume that is made up of red blood cells is called the hematocrit and is a common measure of red blood cell levels.

In a normal blood smear, red blood cells will appear as regular, round cells with a pale center while variations in the size or shape of these cells may suggest a blood disorder as shown in **Fig. 1.2a and b respectively.**

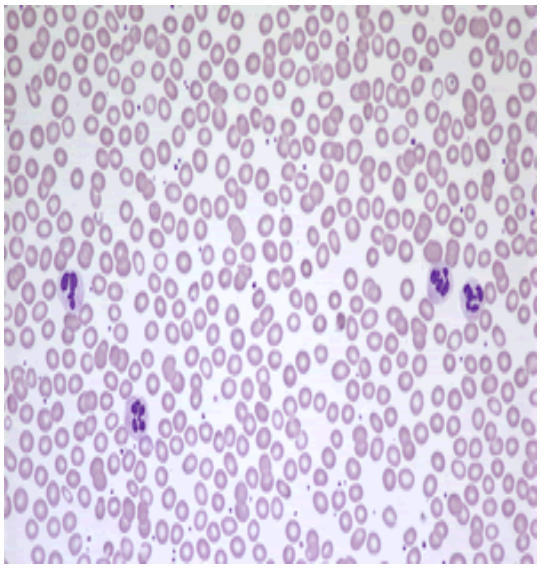


Fig. 1.2a: Normal blood smear [7]

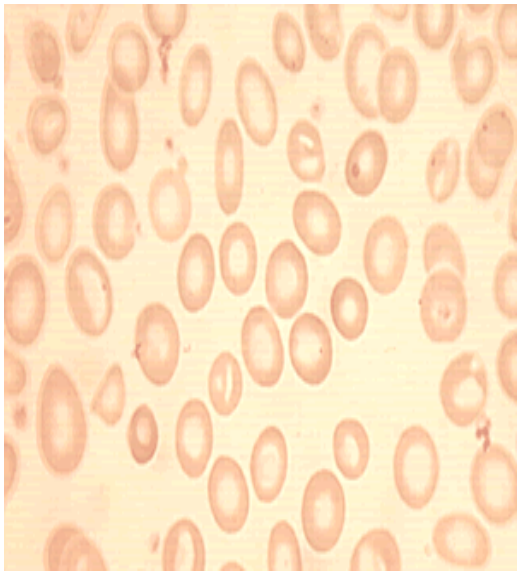


Fig. 1.2b: Abnormal blood smear [8]

Approximately one third of the mass of a mammalian red blood cell is hemoglobin. Its major function is to carry oxygen from the lungs through the arteries to the tissues and help to carry carbon dioxide through the veins back to the lungs. The process whereby hemoglobin performs this essential physiological role is characterized by a cooperative interaction among its constituent subunits.

1.5.2 Hemoglobin structure

The hemoglobin molecule is nearly spherical with a diameter of 55 angstroms and made up of four polypeptide chains: two alpha chains of 141 amino acid residues each and two beta chains of 146 amino acid residues each [9]. The alpha and beta chains have different sequences of amino acids but fold up to form similar three-dimensional structures.

1.5.3 Oxygen Transport

Oxygen has a limited solubility in blood with only about 1.5 % of oxygen carried in the blood is dissolved in the blood plasma. The majority of oxygen is transported in blood by hemoglobin.

The reaction of oxygen with hemoglobin is temporary and completely reversible

$$\text{Hemoglobin} + \text{Oxygen} \rightleftharpoons \text{Oxyhemoglobin}$$

$$Hb + O_2 \rightleftharpoons HbO_2$$

Each hemoglobin molecule contains 4 iron molecules that can each bind an O_2 molecule. Therefore each hemoglobin molecule is capable of carrying 4 O_2 molecules.

$$Hb + 4O_2 \rightleftharpoons HbO_8$$

Hemoglobin loads/unloads one O_2 molecule at a time so hemoglobin, Hb can exist as Hb (deoxyhemoglobin), HbO_2, HbO_4, HbO_6 or HbO_8 (fully saturated oxyhemoglobin).

The binding of oxygen to hemoglobin is dependent on the partial pressure of oxygen. Oxygen combines with hemoglobin in oxygen-rich situations, such as the pulmonary capillaries. Oxygen is released by hemoglobin in oxygen-deficient situations, such as exercising muscle. When oxygen loaded blood reaches exercising tissues there is another large difference in partial pressures (high in blood, low in tissues), so oxygen diffuses into the tissues.

1.5.4 Co-operative binding of oxygen by hemoglobin

Binding of oxygen to hemoglobin is known as co-operative binding because the binding of successive O_2 molecules facilitates binding of the next. Hemoglobin can exist in two conformational states:

Relaxed (R) state - this state corresponds to the quaternary structure of oxyhemoglobin and favours oxygen binding

Tense (T) state - this state corresponds to the quaternary structure of deoxyhemoglobin and has a lower binding affinity for oxygen

Binding of oxygen causes a change in the conformational state of the hemoglobin molecule bringing about a change in the position of the heme groups. Oxygen is transported as molecular O_2 and is not ionically bound to the iron molecule. It is this very loose, reversible binding that enables oxygen to be taken up and released so readily.

2. FEATURES OF SCD

Sickle cell disease or sickle cell anemia is a genetic disease in which red blood cells may change shape under certain circumstances. This causes the cells to become stuck in capillaries which deprives the downstream tissues of oxygen and causes ischemia (an inadequate flow of blood to a part of the body, caused by constriction or blockage of the blood vessels supplying it) and infarction (the death of part or the whole of an organ that occurs when the artery carrying its blood supply is obstructed by a blood clot known as thrombosis or by an embolus). The disease usually occurs in periodic painful attacks, eventually leading to damage of internal organs, stroke or anemia and usually resulting in decreased lifespan. It is common in people from countries with a high incidence of malaria, and especially in West Africa.

Anemia in SCD patients is a condition that is characterized by a reduction in the oxygen carrying capacity of the blood. This reduction is caused by inadequate levels of hemoglobin, inadequate numbers of erythrocytes (low hematocrit) or both. Symptoms of anemia are variable but may include shortness of breath, fatigue, increased heart rate, pale skin, low blood pressure.

Patients have baseline anemia that varies in severity, with typical hemoglobin levels of 7-9 mg/dl. Often white blood cell counts are elevated simply due to marrow hyperactivity. Reticulocyte (immature RBC) counts are elevated, reflecting new red blood cells replacing the rapidly cleared older cells. Thus, red blood cell life span is markedly reduced in this disease.

There are several situations that can lead to this state. The causes of anemia include one or more of the following causes such as folic acid deficiency, hemorrhage, hemolysis, bone marrow failure and kidney disease.

2.1 Vasoocclusive crises

Vasoocclusive crises are caused by sickled red blood cells that obstruct capillaries and restrict blood flow to an organ. Vasoocclusive pain is the most common problem experienced by patients with SCD and the most frequent reason for emergency department and hospital admission [10, 11]. The painful episode is time limited and is

followed by a return to the patient's usual baseline. Ischemia, infarction and inflammation may be responsible for initiating the painful episode and the tissues must contain nociceptors for the vasoocclusive event to be perceived by the patient as being painful.

The hallmark of sickle cell-related pain is its variability. The frequency, location, duration, severity and character of pain differ both within and among patients. The painful event is not synonymous with vasoocclusion [12]. Pain can be precipitated by many events including cold, dehydration, infection, stress and menses. However, the majority of painful episodes have no as yet identifiable trigger(s).

2.1.1 Types of vasoocclusion

Aplastic crisis is an acute worsening of the patient's baseline anemia producing pallor and fatigue. This crisis directly affects erythropoiesis (production of red blood cells). Reticulocyte counts drop dramatically during the illness and the rapid turnover of red cells leads to the drop in hemoglobin. Most patients can be managed supportively but some need blood transfusion.

Splenic sequestration crisis is an acute enlargement of the spleen causing pain. Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected. It is usually infarcted before the end of childhood in individuals suffering from sickle cell anemia and increases the risk of infection from encapsulated organisms. Liver failure may also occur with time.

Bone is also a common target of vasoocclusive damage, especially when the bone is particularly weight-bearing. Such damage may result in avascular necrosis (especially of the femur) and bone deterioration. The pain experienced by sickle-cell patients is also due to the bone ischemia.

A recognized type of sickle crisis is the acute chest crisis, a condition characterized by fever, chest pain, and pulmonary infiltrate on chest x-ray.

2.1.2 Complications of SCD

Sickle cell anemia can lead to various complications, including:

- Overwhelming post (auto) splenectomy infection.
- Stroke
- Cholelithiasis and cholecystitis (gallstones)
- Avascular necrosis (aseptic bone necrosis) of the hip
- Priapism and infarction of the penis (in men)
- Osteomyelitis (bacterial bone infection)
- Opioid addiction

2.2 Kinetics and mechanism of gel formation

A large variety of techniques have been used to monitor the Hb S polymerization process such as the linear birefringence [13 - 16].

Polymerization kinetics is marked by a delay period during which no aggregation is detected by a variety of physical techniques followed by an explosive, autocatalytic formation of polymer [13, 17]. The rate of polymerization is dependent on concentration and stochastic variations in the time at which polymerization occurs in small volumes [18]. The delay time depends reciprocally on the hemoglobin S concentration to the power which varies from about 15 at 0.35g/cc [18] to as high as 35 to 50 at 0.25g/cc [13]. The delay time is also very sensitive to temperature and to most solution conditions.

The early findings of an increased blood viscosity and the observation of a sharp increase in the number of sickled red cells at venous tensions proposes that an increase in blood viscosity slows blood flow in the microcirculation, initiating a cycle of increased oxygen extraction, additional sickling and further viscosity increase, the final result being 'masses of sickled erythrocytes' solid enough to occlude vessels and result in the 'thrombotic' episodes characteristic of the disease.

2.3 Therapeutic options

Blood transfusion, bone transplant and hydroxyurea are a few therapeutic options but they are done both empirically and symptomatically to alleviate the pains and consequences of the vasooclusion experienced by SCD patients. Research is still ongoing for the following therapeutic options to become very useful in the near future in the management of Sickle Cell Disorder.

i. Short chain fatty acids: The use of short chain fatty acids like butyrate interferes positively with the modulation of Hb F levels. Currently, there are still discrepancies on the regimen that is both tolerable for the patient given the desired Hb F levels [19, 20].

ii. Modifiers of oxygen affinity: Agents such as nitric oxide increases the oxygen affinity of Hb S both in vivo and in vitro but the clinical efficiency and long term toxicity is yet unknown [21, 22].
iii. Membrane active drugs: Clotrimazole and magnesium have been investigated with regards to strategies for the prevention of dehydration of sickle red cells. It has also been suggested that magnesium supplement may be effective in reducing the frequency of painful crises [23].
iv. Drugs interfering with adherence: Agents that block hydrophobic adhesive reactions are predicted to reduce the adhesion of sickled red cells to endothelium. This has been shown to significantly reduce analgesic requirements, pain intensity and duration of hospital stay in some SCD patients with painful crises [24].
v. Gene therapy: Since gene therapy is now a reality for certain genetic defects [25], it is hoped that the transfer of a normal β-globin gene along with key regulatory sequences to correct the genetic basis for SCD in autologous stem cells would be the ideal cure for SCD.

3. REVIEW OF MODEL DEVELOPMENT

3.1 A theoretical model for blood flow in small vessels

This model describes blood flow in small vessels of the circulatory system of humans with the inclusion of suspended red cells (the bane of the sickle cell disease). It is an improvement on previous models of blood flow as a single phase homogeneous Newtonian viscous fluid which was previously used [26 - 29].

In this model, Srivastava [30] considered the axisymmetric flow of blood in a uniform circular tube of radius, R. Blood is represented by a two-fluid model consisting of a core region (central layer) of suspension of all the erythrocytes assumed to be a particle-fluid mixture (that is, suspension of red cells in plasma) of radius, R_1 and a peripheral layer of plasma (Newtonian fluid) of thickness $(R - R_1)$ as shown in **Fig. 3.1 below.**

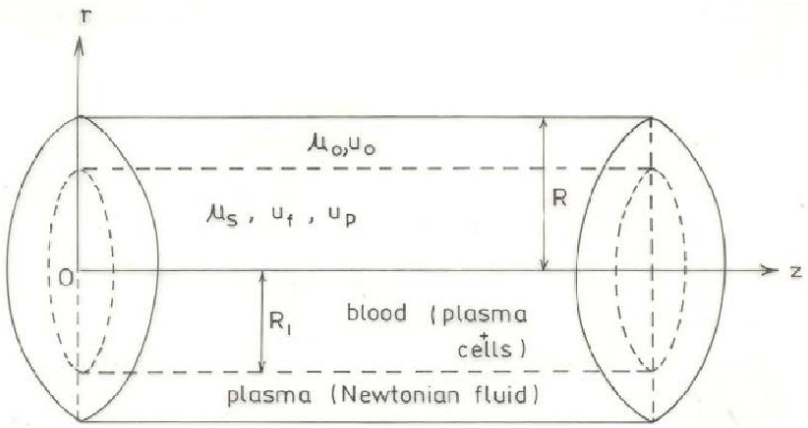


FIG**. 3.1: Flow geometry in small vessels [30]**

From the foregoing, the assumptions considered are:
i. The red cell is a rigid, spherical particle.
ii. The cell-cell interaction is neglected
iii. Interaction between the two phases is according to Stoke's drag law modified to account for finite particulate volume fraction
iv. The volume fraction occupied by red cells (hematocrit) in blood is taken as constant
v. The Brownian motion of red cells is neglected
vi. To neglect any controlling action of the values, the artery wall is assumed to be rigid and its length infinite
vii. The flow is steady and
viii. There is rotational symmetry of the flow

In view of the above assumptions and using a continuum approach,
(Rate of increase of flow) = (Rate of flow in) – (Rate of flow out)
The equations describing the steady flow of a particle-fluid system as expressed by Srivastava et al [31] are:

$$\frac{dp}{dz} = \frac{\mu_0}{r}\frac{\partial}{\partial r}\left(r\frac{\partial}{\partial r}\right)U_0, \qquad R_1 < r < R \tag{1}$$

$$(1-C)\frac{dp}{dz} = (1-C)\frac{\mu_s(C)}{r}\frac{\partial}{\partial r}\left(r\frac{\partial}{\partial r}\right)u_f + CS(u_p - u_f), \qquad 0 \le r \le R_1 \tag{2}$$

$$C\frac{dp}{dz} = CS(u_f - u_p), \qquad 0 \le r \le R_1 \tag{3}$$

where:
(r,z) = (Radial, Axial) co-ordinates
(u_f, u_p) = axial velocity of (fluid, particle) in the core region ($0 \le r \le R_1$)
(μ_0, u_0) = (viscosity, fluid velocity) in the peripheral region ($R_1 \le r \le R$)
$\mu_s \cong \mu_s(C) =$ Suspension viscosity in the core region
C = constant volume fraction density of the particle (Hematocrit)
S = drag co-efficient of interaction between the two phases (fluid and particle)
The expression for the drag co-efficient of interaction, S and empirical relation for the viscosity of suspension μ_s are given empirically [32 - 33] as:

$$S = \frac{9}{2}\frac{\mu_0}{a_0^2}\frac{\left[4 + 3(8C - 3C^2)^{1/2} + 3C\right]}{(2 - 3C)^2}, \tag{4}$$

$$\mu_s = \frac{\mu_0}{1 - qC}; \quad q = 0.07exp\left[2.49C + \frac{1107K}{T}exp(-1.69C)\right] \tag{5}$$

where:
a_0 = radius of a particle
T = temperature in Kelvin.
The boundary conditions are the standard no-slip conditions of velocities and shear stresses at the tube wall and the interface and are given as:

$$u_0 = 0 \quad \text{at} \quad r = R, \tag{6}$$

$$u_0 = u_f \quad \text{and} \quad \tau_0 = \tau_f \quad \text{at} \quad r = R_1, \tag{7}$$

$$\frac{\partial U_f}{\partial r} = \frac{\partial U_p}{\partial r} = 0 \quad at\ r = 0 \tag{8}$$

With $\tau_0 = \mu_0 \frac{\partial U_0}{\partial r}$ and
$\tau_f = (1 - C)\mu_s \partial U_f/\partial r$
as the shear stresses of the peripheral and central layers respectively

The expressions for the velocities u_0, u_f and u_p obtained as the solutions of equations (1) – (3), subject to the boundary conditions (6) – (8), are given as:

$$u_0 = -\frac{R^2}{4\mu_0}\frac{dp}{dz}\left\{1 - \left(\frac{r}{R}\right)^2\right\}, \qquad R_1 \le r \le R \tag{9}$$

$$u_f = -\frac{R^2}{4(1 - C)\mu_0}\frac{dp}{dz}\left\{\mu'\left[\left(\frac{R_1}{R}\right)^2 - \left(\frac{r}{R}\right)^2\right] + (1 - C)\left[1 - \left(\frac{R_1}{R}\right)^2\right]\right\}, 0 \le r \le R_1 \tag{10}$$

$$u_p = -\frac{R^2}{4(1-C)\mu_0}\left\{\frac{dp}{dz}\left[\mu'\left(\frac{R_1}{R}\right)^2 - \left(\frac{r}{R}\right)^2\right] + (1 - C)\left[1 - \left(\frac{R_1}{R}\right)^2 + \frac{4(1-C)\mu_0}{SR^2}\right]\right\}; \quad 0 \le r \le R_1 \tag{11}$$

where:
$\mu' = \mu_0/\mu_s$
The flow flux (volumetric flow rate) is now calculated as:

$$Q = Q_0 + Q_f + Q_p, \tag{12}$$

where:

$$Q_0 = 2\pi \int_{R_1} ru_0\,dr, \quad Q_f = 2\pi(1 - C)\int_0 ru_f\,dr \quad and \quad Q_p = 2\pi C\int_0^{R_1} ru_p\,dr$$

Using eqns 9 - 11, the expression for flow flux is:

$$Q = -\frac{\pi R^4}{8(1-C)\mu_0}\frac{dp}{dz}\left\{(1-C)\left[1-\left(\frac{R_1}{R}\right)^4\right]+\mu'\left(\frac{R_1}{R}\right)^4+\eta^2\left(\frac{R_1}{R}\right)^2\right\}, \quad (13)$$

where: $\eta^2 = 8C(1-C)\mu_0/SR^2$, is a non-dimensional suspension parameter. The use of the fact that the total flux is equal to the sum of the fluxes across the two regions (peripheral and core) determines the relation of Haynes and Bugliarello et al [34 - 35].

$$R_1 = \alpha R. \quad (14)$$

Application of eqn. 13 into 14, yields the expression for the effective (apparent) viscosity as:

$$\mu_e = \frac{(1-C)\mu_0}{(1-C)(1-\alpha^4)+\mu'\alpha^4+\eta^2\alpha^2} \quad (15)$$

When $R_1 = R$ (i.e. in the absence of the peripheral layer), above results reduces to:

$$\mu_{es} = \frac{(1-C)\mu_0}{\mu'+\eta^2}. \quad (16)$$

which is the case of a single layered model of a particle-fluid suspension. Also, in the absence of the particles (i.e. C = 0), the core fluid changes to the same fluid as in the peripheral region and thus the role of the peripheral layer automatically disappears. In addition, when core mixture behaves as a single-phase fluid of constant viscosity (i.e. $\mu_s = \mu_1 \neq \mu_0$), the expression is obtained as:

$$\mu_{eb} = \frac{\mu_0}{1-\alpha^4+\mu'\alpha^4}. \quad (17)$$

Eqn. 17 recovers the result when μ_0 = 1cp.

Results show that the effective viscosity deviates from experimental values with increasing hematocrit and also with the vessel size. Also, the Fahreus-Lindqvist effect was observed in which apparent viscosity of blood decreases with decreasing diameter of blood vessel. Figures 3.1 and 3.2 display graphically the axial velocities (u_f, u_p, u_o) computed from eqns. 16 – 17. It was observed that red blood cells velocities at the tube axis assumes higher magnitude than the plasma velocity but the difference in their magnitude decreases with increasing radial co-ordinate, r towards the interface. At the interface, the plasma velocity coincides with the blood velocity. Also, the volumetric flow rate, Q vs pressure gradient, –dp/dz computed from eqn. 13 of the model at 20% and 40% hematocrit compared with experimentally tested model of Bugliarello and Sevilla [35] is shown in Fig. 3.3.

The results of Srivastava [30] (shown in Figs. 3.1 and 3.2 below) at 20% and 40% hematocrit show that erythrocyte velocity at the tube axis assumes higher magnitude than the plasma velocity but the difference in their magnitudes decreases with increasing radial coordinate r towards the interface and at the interface, the plasma velocity coincides with the blood velocity when comparing with previous models.

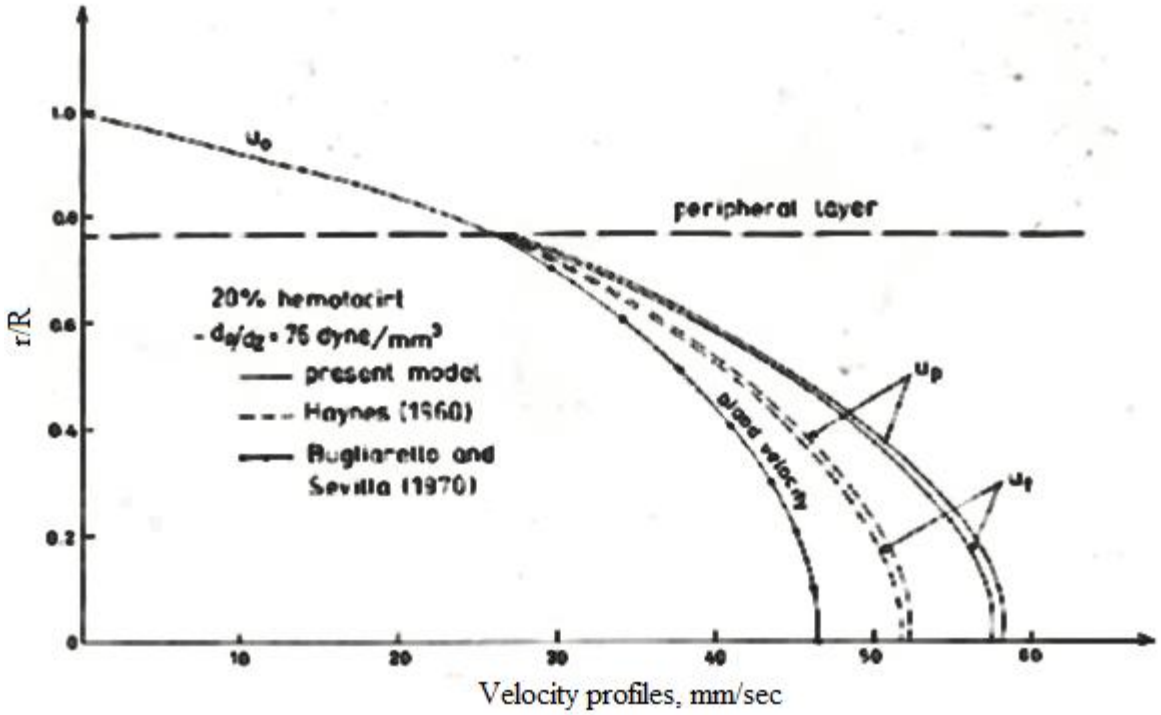


Fig. 3.1: Velocity profiles at 20% hematocrit in a 40µm diameter vessel [30].

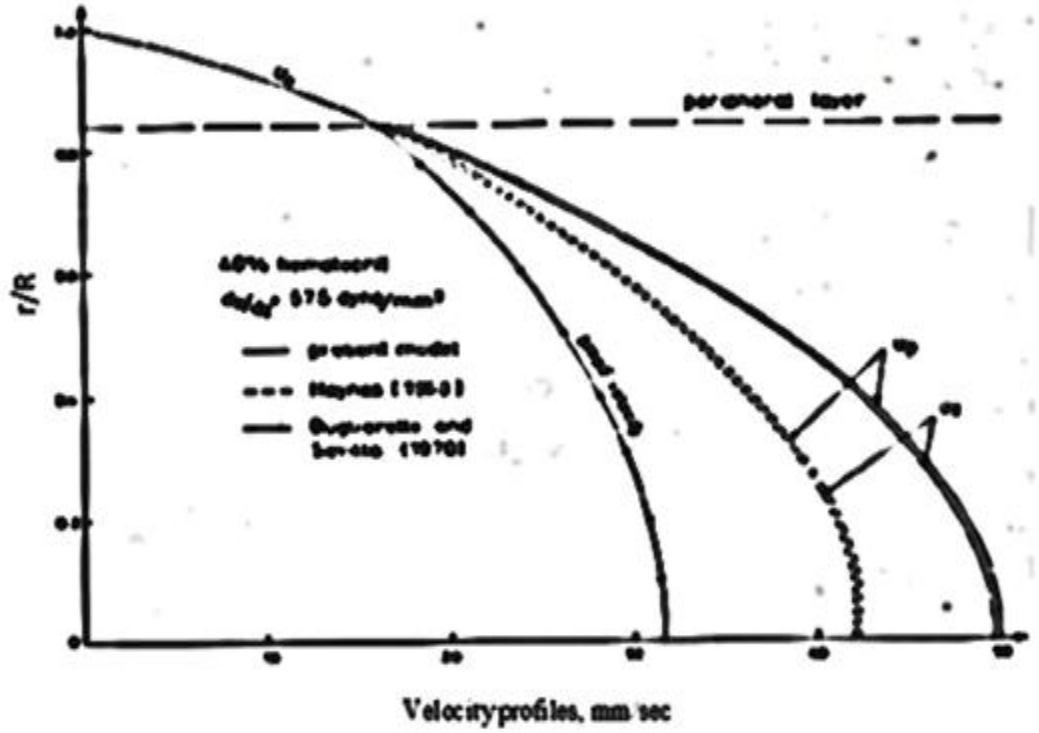


Fig. 3.2: Velocity profiles at 40% hematocrit in a 40µm diameter vessel [30].

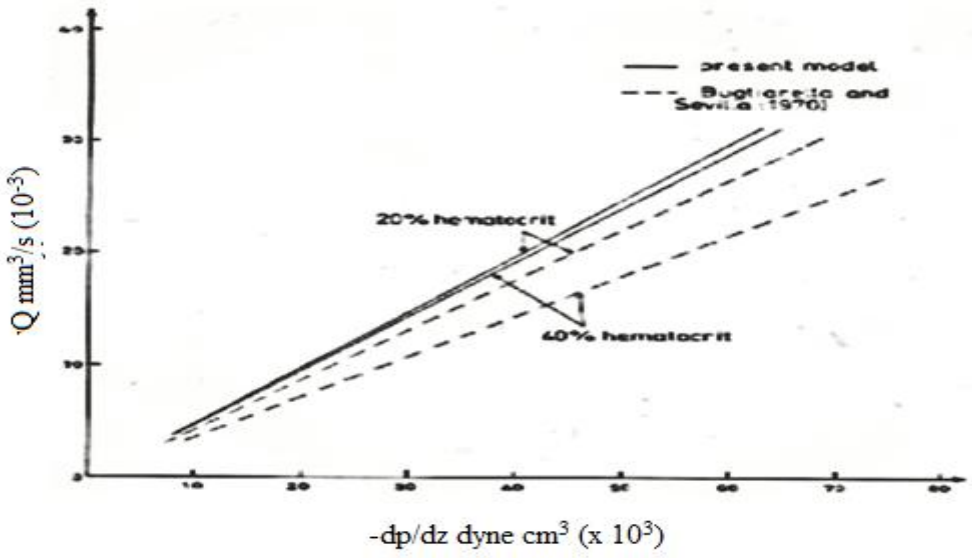


Fig. 3.3: Pressure flow rate relationship in a 40µm diameter vessel for different hematocrit [30].

3.2 Evaluation of Red cell membrane elasticity and viscosity

This model [36] attempted the evaluation of the cell membrane elasticity which is of paramount importance since the polymerization of the Hb S affects the red cell membrane and its ability to deform (deformability).

Assumptions:

- Parallelepiped cell model with length, L and width, W and negligible thickness, δ.
- Optical tweezers dragged the red cell with six constant velocities, V
- The fluid is human AB plasma with viscosity, η.
- One surface stands still (cell surface) and the other moves with a velocity, V in a viscous fluid with viscocity, η.

Hence,

$$F_{Drag} = \eta \left(\frac{W L_0}{Z_{eq}} \right) V \qquad (18)$$

With $\frac{1}{Z_{eq}} = \frac{1}{Z_1} + \frac{1}{Z_2}$

Z_1, Z_2 = cell distance from the Neubauer chamber and from the cover slip of the Neubauer chamber respectively

Z_{eq} = equivalent depth

Assuming the overall cell elastic response to an applied force, F is given by;

$$F_{Elastic} = \frac{\mu W}{L_0 \Delta L} \qquad (19)$$

where, µ = overall elasticity

$\Delta L(L - L_0)$ = cell length deformation, adopting L_0 as the cell length in the absence of any force.

At equilibrium, the elastic force cancels the drag force and the cell length is given by:

$$L = L_0 + \left(\frac{\eta L_0^2}{\mu Z_{eq}} \right) V \qquad \text{which is independent of cell width, W} \qquad (20)$$

Therefore, the measurement of the cell length as a function of the drag velocity is used to extract a value for µ, once the plasma viscosity, η, the initial length, L_0 and Z_{eq} are known. The slope of this curve, $\frac{\eta L_0^2}{\mu Z_{eq}}$ is also a function of the equivalent depth Z_{eq} and can be used to test this model.

Red blood cell length vs drag velocity.

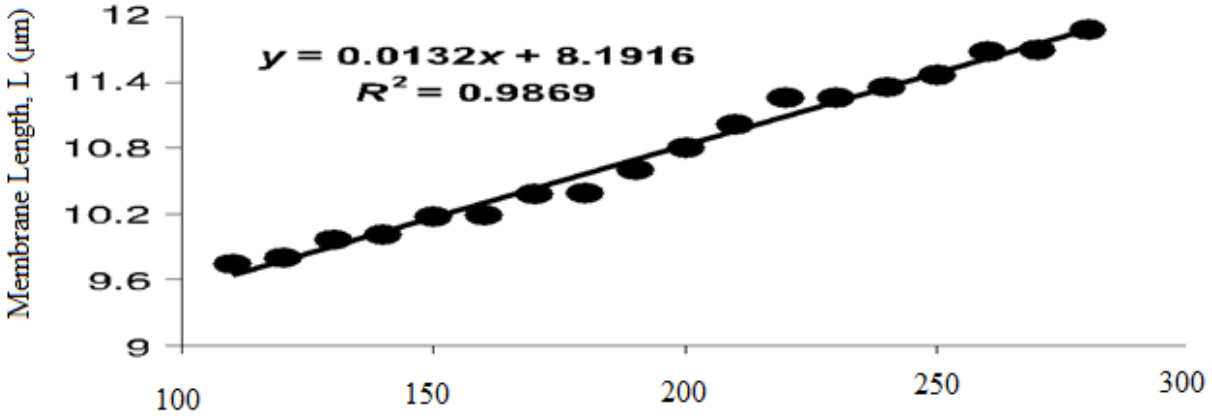
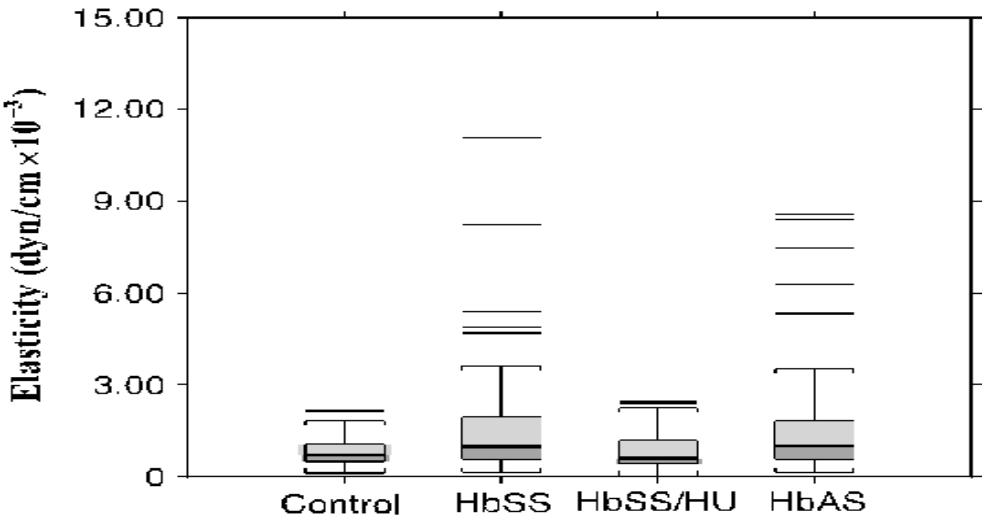


Fig. 3.4: Red blood cell length plotted as a function of the drag velocity [36].

Results showed that the deformability of RBC was lower in subjects with Hb S than normal controls. On the other hand, the RBC deformability of HB S homozygous taking hydroxyurea was similar to that observed in the controls. Patients with sickle cell trait did not differ significantly from Hb SS.

The results also showed Hb S deformability presented a widespread distribution compared with normal control cells **(Fig. 3.5A). The cell deformability data did not have a bimodal distribution but the dispersion was high and some cells escape from the majority (Fig. 3.5B)**

(A)



(B)

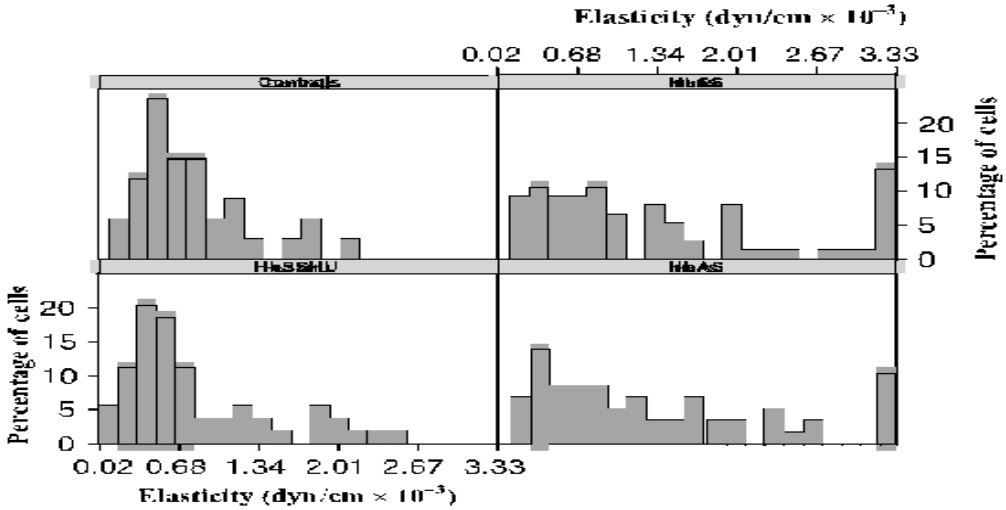


Fig 3.5: Distribution of red cell elasticity in all subjects: A: Box plot without liners B: Histogram without the outline cells [36].

3.3 Quantifying the rheological and hemodynamic characteristics of Sickle Cell Anemia

This model was considered for this study because the mechanical properties of the red cell membrane was determined in the model [37] to show that the adhesive interactions between the sickle cells and the endothelial ligands play a crucial role in triggering the vasoocclusion phenomenon in straight vessels.

Here, the RBC was developed within the framework of the dissipative particle dynamics (DPD) method which is a particle-based method that is widely used for simulation of soft matter at the mesoscopic level. Each DPD particle represents a virtual cluster of atoms or molecules rather than an individual atom [37].

In this model, the RBC membrane is represented by a 2D triangulated network with N_v = 500 vertices where each vertex is represented by a DPD particle. For each single cell, the free energy is defined by:

$$V_{rbc} = V_s + V_b + V_a + V_v \quad (21)$$

where, V_s = viscoelastic bond interaction between the cell vertices such that proper membrane mechanical properties can be imposed.

V_b = binding energy of the cell membrane

V_a, V_v = area and volume constraints to mimic the incompressibility of the lipid bilayer and the intracellular cytosol.

The direction along the thickness of RBC is the z direction and the plane determined by the two long axes is defined as x-y plane. The letters A, B, C and D indicate the four points with the maximum/minimum values in the x/y directions.

Assuming no cell-cell interaction and that the sickle red cell vertices can interact with the endothelial ligands within an interaction distance, d_{on}. Then for each time step, Δt, transient bonds can be formed between the cell vertices and the endothelial ligands with probability,

$$P_{on} = 1 - e^{-k_{on}\Delta t} \quad (22)$$

And the existing bonds can be ruptured with probability,

$$P_{off} = 1 - e^{-k_{off}\Delta t} \quad (23)$$

where, k_{on} and k_{off} are reaction rates defined as:

$$k_{on} = k_{on}^0 exp\left(-\frac{\sigma_{on}(l-l_0)^2}{2k_BT}\right) \quad (24)$$

$$k_{off} = k_{off}^0 exp\left(\frac{\sigma_{off}(l-l_0)^2}{2k_BT}\right) \quad (25)$$

where, σ_{on} and σ_{off} = effective formation and rupture strengths respectively.

For existing bonds, the force between receptor and ligands is defined by:

$$F(l) = 2k_s(l - l_0) \quad (26)$$

where, k_s = spring constant

l_0 = equilibrium length

The degree of distortion can be identified by an eigenvalue analysis of the gyration tensor defined by:

$$G_{mn} = \frac{1}{N_v}\sum_i \left(r_m^i - r_m^c\right)\left(r_n^i - r_n^c\right) \quad (27)$$

where, r^i = the RBC vertex coordinates

r^c = the center of the mass and m, n can be x, y or z.

The three eigenvalues obtained from the gyration tensor are denoted by λ_1, λ_2, λ_3 where $\lambda_1 < \lambda_2 < \lambda_3$. The ASF (Asphericity Shape Factor) and ESF (Elliptical Shape Factor) are therefore defined as:

$$ASF = \frac{[(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2]}{2R_g^4} \quad (28)$$

where R_g = radius of gyration defined by $R_g^2 = \lambda_1 + \lambda_2 + \lambda_3$ (29)

$$ESF = \frac{\lambda_3}{\lambda_2} \quad (30)$$

Results show that while the ASF measures the deviation of the RBC from a perfect sphere shape, the ESF measures the degree of distortion on the x-y plane. The figure below shows plots of the ASF and ESF for the three types of cells constructed. The granular cells show characteristics similar to those of healthy cell and the elongated cell exhibits the largest deviation from the perfect biconcave shape (Fig. 3.6). The different morphologies obtained here, conformed to similar morphological analysis previously conducted on the medical image of different sickle cells on 2D plane, circular and elliptical shape factors computed for granular- and sickle-shaped cells.

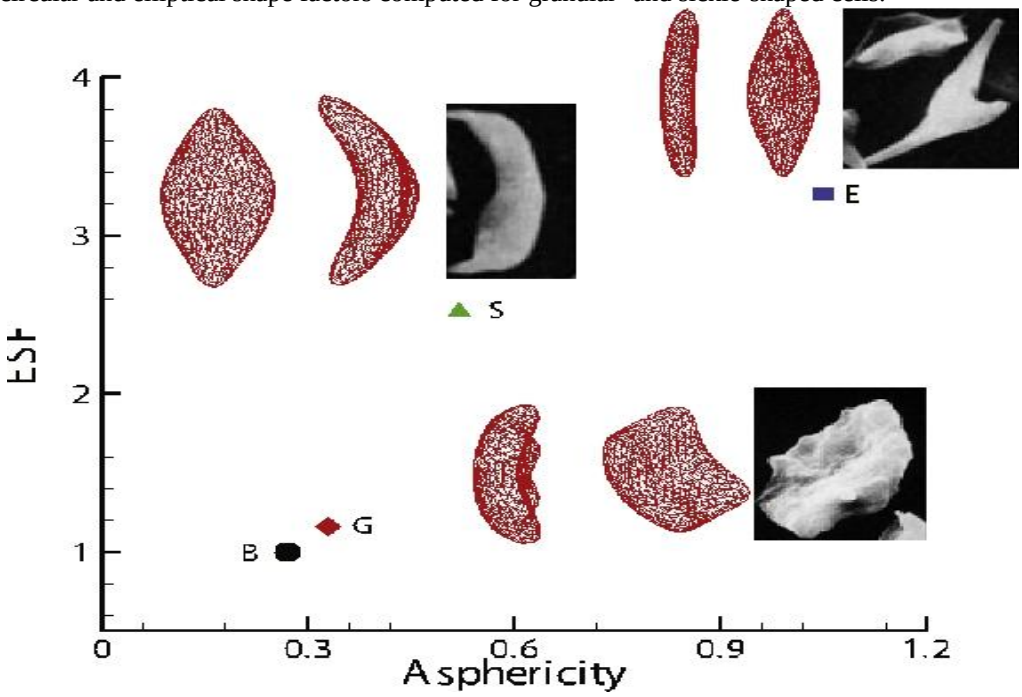


Fig. 3.6: ASF and ESF for the different shapes of sickle cells [37].

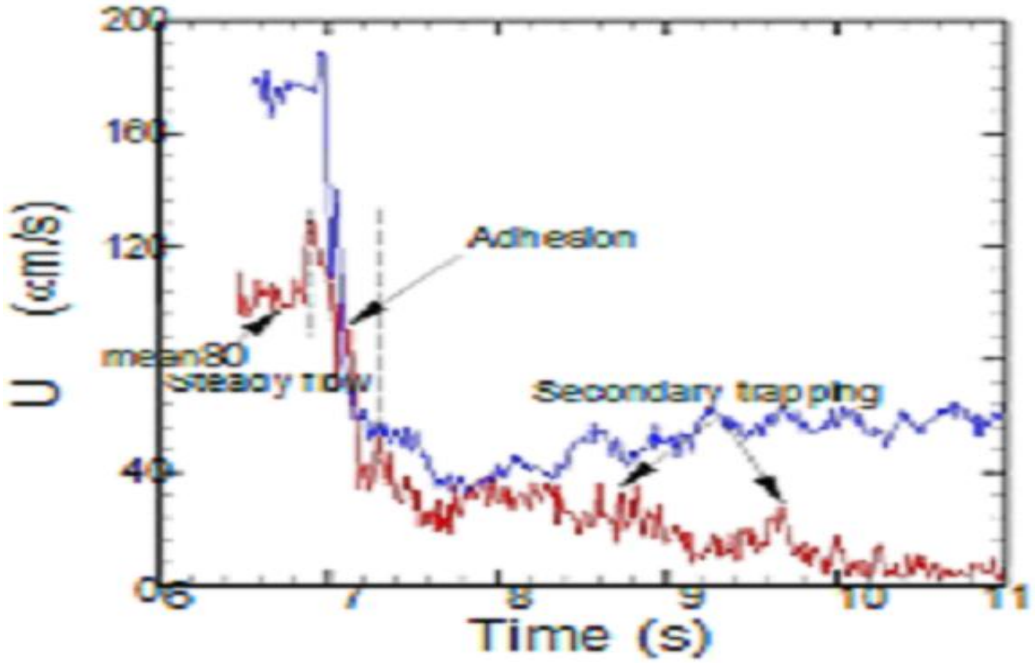


Fig 3.7: Mean velocity of sickle blood at different stages of the adhesion dynamics at pressure drops of 8.3 X 10^4 and 1.35 X 10^5 pa/m [37].

3.4 Analysis of mechanical behavior of red cell membrane in SCD

In this section of the review, deformability as a consequence of several mechanical and rheological properties like internal viscosity, membrane elasticity and viscosity and surface-volume ratio were explored using the work of Demeke [38] where the visco-elastic behavior of red blood cell membrane in normal and in sickle cell disease was investigated.

Consider a red blood cell, RBC, to compose of an inner liquid (hemoglobin solution) and a membrane (boundary of the cell) where the resistance of the inner liquid to external stress is small and negligible.

Assuming that the viscous behavior is dominated by the cell interior, the interior of the cell is modeled as a Voigt visco-elastic solid with parameters for the viscous and elastic modulus while the membrane is assigned an elastic shear modulus. Thus, the generalized Voigt viscoelastic solid model can be thought as a 'mixture' in the following sense,

$$T_{ij} = -P\delta_{ij} + T^{e}_{ij} + T^{v}_{ij} \qquad (31)$$

where

T^{e}_{ij} = stress associated with an elastic component that can be expressed as a function of strain

T^{v}_{ij} = stress associated with viscous fluid and depends on the rate of deformation

$-P\delta_{ij}$ = the reaction stress due to incompressibility

A general viscoelastic constitutive equation for erythrocyte membrane may be written in terms of principal strains and stresses. This implies no loss of generality, assuming that the membrane is isotropic in its own plane. Let deformation of the membrane as described by initial lengths dx_1, dx_2, and current lengths dy_1, dy_2 in a Cartesian coordinate system parallel (tangent) to the local principal direction. The extension ratios λ_1 and λ_2 are defined as ratios of final to initial lengths

$$\lambda_1 = \frac{dy_1}{dx_1} \quad \text{and} \quad \lambda_2 = \frac{dy_2}{dx_2} \qquad (32)$$

The Green's strain tensor for large deformations is defined by:

$$e_{11} = \frac{1}{2}(\lambda_1^2 - 1)\ , \qquad e_{22} = \frac{1}{2}(\lambda_2^2 - 1) \qquad (33)$$

Relating the stress to strain via W, the strain energy function, the principal membrane tensions are thus expressed by

$$T_i = \frac{1}{\lambda_1\lambda_2}\lambda_i\frac{\partial W}{\partial \lambda_i} \qquad (i = 1,2) \tag{34}$$

Assuming that the membrane behaves like a neo-Hookean solid material, then the strain energy for a 2-D neo-Hookean membrane is given as:

$$W = \frac{Gh}{2}\left(I_1 + \frac{1}{I_2 + 1}\right) \tag{35}$$

where G = shear modulus of elasticity of the membrane
h = membrane thickness

The 2-D strain invariant I_1 and I_2 are defined by [Skalak et al, 1973]:

$$I_1 = \lambda_1^2 + \lambda_2^2 - 2 \qquad and \qquad I_2 = \lambda_1^2\lambda_2^2 - 1 \tag{36}$$

where λ_1 and λ_2 are the principal stretch ratios as defined in eqn 32. The tensions T_1 and T_2 along the principal directions using eqns 34, 35 and 36 are:

$$T_1 = \frac{Gh}{\lambda_1\lambda_2}\left(\lambda_1^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right) \tag{37}$$

$$T_2 = \frac{Gh}{\lambda_1\lambda_2}\left(\lambda_2^2 - \frac{1}{(\lambda_2\lambda_2)^2}\right) \tag{38}$$

While the viscous strain term is given as:

$$T_{ij}^v = 2\eta_m V_{ij} \tag{39}$$

where η_m = co-efficient of viscosity
V_{ij} = rate of deformation tensor.

The difference between the squared lengths in the deformed and undeformed (initial) co-ordinate system is defined as:

$$(ds)^2 - (ds_0)^2 = dy_k\,dy_k - dx_k\,dx_k = 2e_{ij}\,dx_i\,dx_j \tag{40}$$

Taking the time derivatives of both sides of eqn. 40 yields

$$\frac{d}{dt}((ds)^2) = 2e_{ij}\,dx_i\,dx_j \tag{41}$$

The facts are that (ds_0), dx_i *and* dx_j are constants. Hence from the right side of eqn. 41, we obtain:

$$\frac{d}{dt}((ds)^2) = \frac{d}{dt}(dy_k\,dy_k) = \left(\frac{\partial v_i}{\partial y_j} + \frac{\partial v_j}{\partial y_i}dy_i\,dy_j\right) = 2V_{ij}\,dy_i\,dy_j \tag{42}$$

where v_i is the *i*th component of the in-plane velocity field, from the combination of eqns 41 and 42, we obtain:

$$v_{ij}dy_i dy_j = e_{kl}dx_k dx_l$$

$$\Longrightarrow V_{ij} = e_{kl}\frac{\partial x_k}{\partial y_i}\frac{\partial x_l}{\partial y_j} \tag{43}$$

Then from eqns. 32, 33 and 43, the components of the rate of deformation tensors are given by

$$V_{11} = \frac{1}{\lambda_1}\frac{d\lambda_1}{dt} \quad , \quad V_{22} = \frac{1}{\lambda_2}\frac{d\lambda_2}{dt} \tag{44}$$

From eqns. 39 and 44,

$$T_{11}^v = \frac{2\eta_m}{\lambda_1}\frac{d\lambda_1}{dt} \quad , \quad T_{22}^v = \frac{2\eta_m}{\lambda_2}\frac{d\lambda_2}{dt} \tag{45}$$

Assuming further that T_{ij}^e from eqn. 31 is the same as T_i in eqn, 34 and using eqns. 37, 38 and 45 in eqn 31, the membrane tensors versus strain and rate of strain undergoing simple extension (in the principal axis system) can be written as:

$$T_{11} = -p + \frac{Gh}{\lambda_1\lambda_2}\left(\lambda_1^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right) + \frac{2\eta_m}{\lambda_1}\frac{d\lambda_1}{dt} \tag{46}$$

$$T_{22} = -p + \frac{Gh}{\lambda_1\lambda_2}\left(\lambda_2^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right) + \frac{2\eta_m}{\lambda_2}\frac{d\lambda_2}{dt} \tag{47}$$

If we assume the element of deformation experiences uniaxial tension, then T_{22} would be zero and the result for T_{11} simplifies to:

$$T_{11} = -\frac{Gh}{\lambda_1\lambda_2}\left(\lambda_2^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right) - \frac{2\eta_m}{\lambda_2}\frac{d\lambda_2}{dt} + \frac{Gh}{\lambda_1\lambda_2}\left(\lambda_1^2 - \frac{1}{(\lambda_1\lambda_2)^2}\right) - \frac{2\eta_m}{\lambda_1}\frac{d\lambda_1}{dt} \quad (48)$$

where the in-plane principal tension and "11" indicates the in-plane direction along the membrane and the unit normal of the plane on which the tension force is acting. Thus, during experiences of the in-plane membrane deformation, the membrane area is constraint, that is , $\lambda_1\lambda_2 = 1$ (where $\lambda_3=1$), in this condition the in-plane shear modulus, $\mu = Gh$ throughout the entire deformation. Therefore eqn. 48 reduces to:

$$T_{11} = \mu\left(\lambda_1^2 - \frac{1}{\lambda_1^2}\right) + \frac{4\eta_m}{\lambda_1}\frac{d\lambda_1}{dx} \quad (49)$$

where μ = shear modulus

η_m = membrane viscosity

Results show that as membrane shear elastic modulus, μ and membrane viscosity, η_m are elevated while the strain rate is decreased (assuming that the Hb S polymerization occurred in sickle cell erythrocytes) the curves of sickle erythrocytes differ from and above the normal erythrocytes as shown in Fig. 3.8 below. These curves clearly show that a movement from curve A to C, the behavior of the cell become more rigid and poorly deformable.

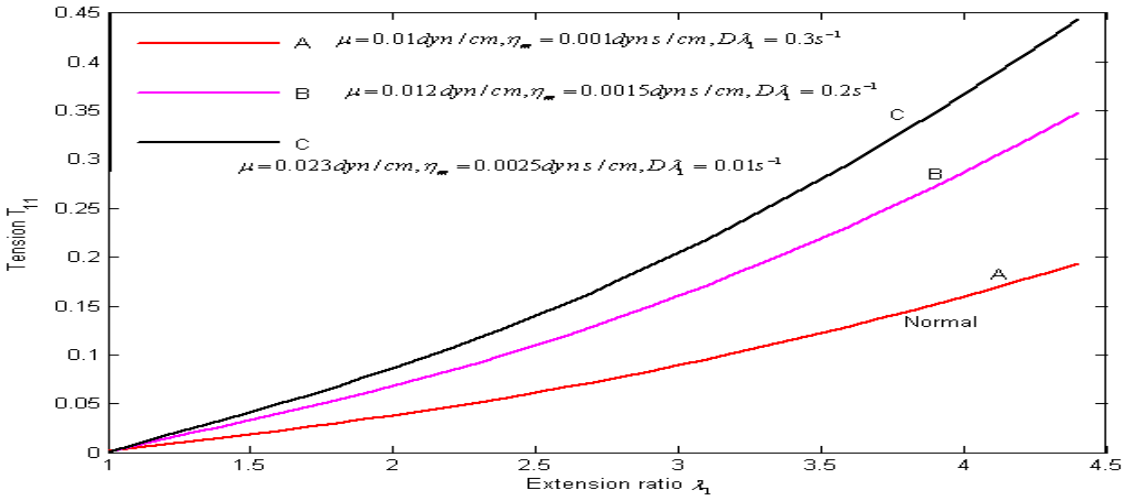


Fig. 3.8: Stress versus strain and rate of strain curves in uniaxial tension. The different curve shows various values of the parameters and strain rate using equation (49) [38]

4. DISCUSSION

While previous models of blood flow considered a single homogeneous fluid, the theoretical model for blood flow in small vessels [30] takes into cognizance the presence of erythrocytes. This obviously is a better model since the RBC (erythrocytes) is directly responsible for blood properties and disease of SCD. Although effective viscosity, velocity profiles and flow rates were numerically analyzed for blood with low hematocrit (≤ 40%) and in vessels up to 70μm yet it has the following limitations:

- It does not give an adequate representation of flow field in very small vessels (4 - 10μm) where the sickling/gelling or polymerization of the hemoglobin is presumed to occur most.
- The individuality of the red blood cells was not put into consideration, that is, the peculiarity of each cell was not investigated.
- The assumption of no RBC in the peripheral layer is not accurate as it completely excludes red cell interaction with the endothelium.
- The assumption that the RBCs are small spherical non-flexible particles is not an accurate approximation because RBCs are indeed flexible and deformable. Otherwise, there would be no difference between the mechanical/elastic properties of normal and sickled red cells. In other words, the deformability of the RBC is the core determinant in the management of sickle cell disorder.

Since the deformability of red blood cells is a critical determinant of blood flow in microcirculation, the elasticity and viscosity of RBC was measured using the more sensitive technique – the laser optical tweezers [36]. Individual cell analysis is necessary to detect differences between normal and abnormal populations. Unlike the micropipette technique previously used which measures only the cell membrane deformability; the optical tweezers measures the

entire cell deformability (that is, overall elasticity) and not only the intrinsic membrane elasticity. This is paramount since the rheological behavior of RBC is determined by both membrane and cytoplasm properties.
The overall elasticity is very sensitive to any change in the cell environment such as osmotic pressure, changes due to molecules attached to the cell surface or hemoglobin defect. The results of this model conform to known facts that the deformability of sickle red cells is lower than that of normal red cells.
Huan [37] attempted to describe the RBC morphology and membrane mechanics in deoxygenated conditions revealing also cell stiffening during deoxygenation. It also reveals how cell morphology affects viscosity. Full occlusion was also recorded with the incorporation of adhesive dynamics model into the simulation. This model has the advantage of:

- Identifying the heterogeneous characteristics of SS-RBCs
- It tries to explain the physiological condition for the occurrence of vasoocclusion
- It captures adhesive interactions of RBCs and endothelium as being contributory to vasoocclusion even in straight vessels of 9μm diameters which is within the range of likely frequent occlusion.
- It captures SS-RBCs suspension in transition from Non-Newtonian to Newtonian flow.
- The effect of different types of SS-RBCs on the flow resistance in the microcirculation was also considered.

The limitations of this model include the following:

- It considers a cluster of cells rather than individual cells.
- The fixed value of bending rigidity of sickle cell under different deoxygenated stages could affect the quality of results obtained.
- The adoption of sickle cell morphology for simulation depending on hematocrit levels (Granular for Hct >35% and spherical for hematocrit < 35%) is an inaccurate choice since cell morphology is determined not only by hematocrit but by the speed and duration of oxygenation [37].

Demeke [38] viewed the cell interior as an incompressible visco-elastic continuum which is a fair approximation of the rigidity of the interior as seen in the protrusions of the SS-RBC upon deoxygenation, that is, if the polymers formed were soluble, there would be no protrusions in the interior. It attempts to account for cell volume loss as a result of the polymerization of protein within the cells of the subcellular organelles. The analysis from this model is consistent as reported on published literature, that both μ and η_m similarly elevated above normal for ISC membrane elasticity due to an irreversible alteration in membrane structure [39].

5. CONCLUSION

Although the location, duration and extent of occlusion in vessels of SCD patients cannot be fully predicted at present, future work will focus on a combination of models that will endeavor to shed more light on this problem. This envisaged model will involve all the factors contributing to Hb S polymerization/abnormal RBC rheology and geometry.
Vasoocclusion may be seen as an extreme of conditions of mechanical, elastic and viscous properties of the cell which is likely associated with extremes of both flow velocity and transit time and may require only a small subpopulation of cells with these extreme characteristics. The degree and rate of polymerization is significant only as far as it causes vasoocclusion.
The goal of therapeutic intervention (aimed at reducing hemoglobin polymerization in the intact sickle erythrocyte) is to decrease the polymerization potential to that associated with the milder sickle cell syndrome, such as, Hb AS (sickle cell trait).
Future model development is needed for predicting blood flow and vessel blockage (vaso-occlusion) in people with sickle cell disorder using real case studies for analysis.

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