

Red Blood Cell Mechanical Stability

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Received 2012

ABSTRACT

It has been well documented that shear forces effective above a certain magnitude under flow conditions causes damage in blood cells. This damage ranges from slight morphological alteration to the destruction of red blood cells (RBC). The hemolytic trauma to RBC can easily be detected by measuring free hemoglobin level in plasma, however there are no standardized protocols to quantitate subhemolytic trauma. Ektacytometry has been used to study the alterations in RBC mechanical properties induced by the application of shear stress at various levels. Additionally, a protocol for measuring the hemolytic threshold as an indicator of subhemolytic damage to RBC has been developed based on ektacytometry. These standardized protocols may find applications in the pre-clinical and clinical evaluation of artificial organs and biomedical devices contacting with blood.

Keywords: Artificial Organs; Blood Damage; Subhemolytic Damage; Ektacytometry

1. Introduction

Red blood cells (RBC) are exposed to mechanical stresses during their “everyday life”, at various levels of the circulatory system. They respond to this effect by changing their shape and orientation to the flow conditions; but they are not overtly damaged by these forces, under physiological conditions. However, if they are exposed to non-physiological conditions, like in case of circulating through artificial organs or devices, they might be affected resulting in deteriorations at various degrees, ranging from slight morphological alterations to the rupture of their membrane resulting in hemolysis [9].

The most effective factors under such conditions include the extremely high shear forces to which RBC are exposed to during their passage through the artificial flow environments [5, 9]. Additionally, turbulence, cavitation, contact of blood cells with artificial surfaces, and collision between cells or between cells and various parts of the device may also damage the cellular components of blood [5, 9]. Shear forces in the human arterial system are in the range of 1-10 Pascal (Pa) [7], while in artificial circulatory environments (e.g., ventricular assist devices, total artificial heart, extracorporeal circulation systems, heart valves, stents, hemodialysis systems) these forces may approach to several hundred Pa [5].

2. Mechanical Trauma to Blood

It is well known for many decades that shear forces exceeding a critical level may cause hemolysis [12]. It has been demonstrated that both magnitude and exposure time are important determinants of the hemolytic effect of shear stress [6]. Hemolytic damage to RBC can easily be quantitated by measuring free hemoglobin in the suspending medium of blood (plasma), which is usually expressed as the ratio to the total hemoglobin content of whole blood. This hemolysis ratio or its normalized forms are widely used in expressing the extent of mechanical trauma to blood and used for the evaluation of artificial circu-

latory environments [9].

However, it should be noted that hemolysis is the end-stage damage to RBC, corresponding to the destruction of the cell. It has been documented that the mechanical impact to RBC under the hemolytic threshold also induce alterations in the cellular properties, at varying degrees determined by the magnitude of the effective forces [9,15]. It has been argued that even physiological shear forces under normal circulatory conditions induce slight changes in RBC properties, which may accumulate during the life-span of RBC (~120 days for human RBC). This “non-lethal” trauma to RBC might be enhanced if they are exposed to the conditions in artificial circulatory environment [9].

Subhemolytic trauma might be manifest by shortened life span [3], increased trapping in spleen [14], morphological alterations [13], or deterioration in mechanical properties of the cells [2]. Subhemolytic trauma is also determined by both the magnitude and duration of the effective shear stresses [2,9].

RBC membrane stability is affected by subhemolytic trauma, resulting in increased mechanical fragility indicated by higher rate of hemolysis during the subsequent exposure to extreme shear forces. Various tests to determine mechanical fragility has been developed and proposed to be used in testing the artificial circulatory environments [4,8]. Mechanical fragility is expressed as percent free hemoglobin following a standardized mechanical stress to RBC suspensions [9]. However, the magnitude of the mechanical impact to which RBC are exposed cannot be standardized or even accurately estimated with the proposed protocols [4,8].

3. Detection of Subhemolytic Damage to Red Blood Cells

Accurate quantification of subhemolytic damage to RBC is important in studies aiming to evaluate the blood damage induce by artificial organs and biomedical devices contacting with blood. Additionally, test protocols and models based on subhemolytic levels of mechanical trauma to RBC may help

designers to improve the safety limits of the artificial circulatory systems, with respect to blood trauma.

It has been demonstrated that a Couette-type viscometer is a useful apparatus to apply shear stresses with well-defined and constant geometry in a wide range of magnitude. Instruments equipped with a laser-diffraction analysis capability in addition to a shearing apparatus have been used to analyze the alterations in the deformability of RBC exposed to subhemolytic levels of shear stress [2,10,11]. Application of 120 Pa of shear stress for periods of 15 to 120 seconds to RBC suspensions resulted in a deterioration in deformability, the degree of impairment in elongation indexes being a function of the duration of the mechanical stress [2]. **Figure 1** demonstrates the shear stress-elongation index curves of RBC suspensions before and after the application of 100 Pa shear stress for 300 s. The impairment of deformability under a standardized shearing protocol has been observed to reflect the “health” of RBC prior to the application of the shear stress; a subhemolytic trauma to RBC (e.g., due to circulating in an artificial organ) relates to a higher level of impairment following the shearing. RBC with altered membrane stability (e.g., due to exposure to lanthanides of various ionic radius) were characterized by different degrees of impairment in deformability following a standard application of shear stress (i.e., 290 Pa for 300 s) [1]. These observations indicate that ektactometry can be used as a standardized, sub-hemolytic mechanical damage test.

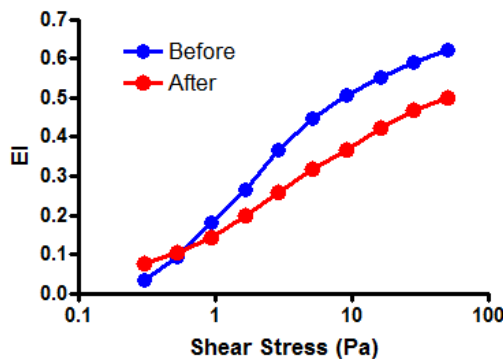


Figure 1. Shear stress-elongation index curves for red blood cell suspensions before and after being exposed to 100 Pascal shear stress for 300 seconds.

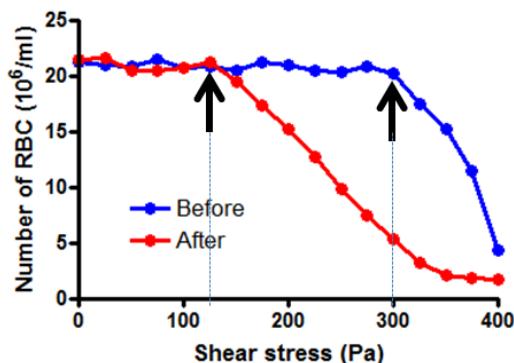


Figure 2. Number of red blood cells (RBC) in suspensions exposed to gradually increasing shear stress before and after the application of 100 Pascal shear stress for 300 seconds. The number of RBC was estimated by the area of elliptical diffraction pattern.

4. Hemolytic Threshold Determined by Ektactometry

Normal RBC can withstand to shear stresses up to 300 Pa, without being hemolysed. However, alterations in membrane stability may significantly reduce this hemolytic threshold. It is worthwhile to measure this hemolytic threshold as an indicator of the accumulated subhemolytic damage to RBC during previous life-span (e.g., in an artificial organ system).

Ektactometry might be used as an easily applicable test protocol to measure the hemolytic threshold, as an indicator of subhemolytic trauma to RBC. The coaxial-cylindrical shearing apparatus of an ektactometer has been used to apply gradually increasing shear stresses (in the range of 0 to 400 Pa) to RBC suspensions and the laser diffraction images have been monitored. It has been recognized that elongation indexes remained relatively constant while the area of the elliptical diffraction pattern has changed more significantly during the course of shear stress application. Preliminary data indicates that the ellipse area is an accurate indicator of the number of RBC in the suspension under test. **Figure 2** demonstrates the alterations in the number of RBC as a function of gradually increasing shear stress. The curve labeled as “Before” reflects the change in the number of RBC in suspensions before being exposed to shear stress, which indicates a sharp decrease in RBC number as shear stress approaches to 300 Pa. This level of shear stress is accepted as the hemolytic threshold. The curve labeled as “After” reflects the same test applied to RBC suspensions following a prior exposure to 100 Pa shear stress for 300 s, indicating a very significantly reduced hemolytic threshold.

5. Conclusion

Ektactometry can be developed into a useful approach in studying the subhemolytic mechanical trauma to blood, to be used in the pre-clinical and clinical evaluation of artificial organs and biomedical devices contacting with blood.

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