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Impact of glycocalyx structure on red cell–red cell affinity in polymer suspensions

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ABSTRACT

A theoretical framework based on macromolecular depletion has been utilized in order to examine the energetics of red blood cell interactions. Three different glycocalyx structures are considered and cell–cell affinities are calculated by superposition of depletion, steric and electrostatic interactions. The theoretical model predicts a non-monotonic dependence of the interaction energies on polymer size. Further, our results indicate that the glycocalyx segment distribution has a large impact on adhesion energies between cells: a linear segment distribution induces the strongest adhesion between cells followed by pseudo-tail and uniform distributions. Our approach confirms the concept of a depletion mechanism for RBC aggregation, and also provides new insights that may eventually help to understand and quantify cellular factors that control red blood cell interactions in health and disease.

1. Introduction

Erythrocytes or red blood cells (RBC) are biconcave cells having a diameter of 6–8 μm and a thickness of about 2 μm with a concentrated hemoglobin solution cytoplasm [1]. RBC make up about 40–50% and 36–46% of the blood volume in men and women, respectively [2]. Being the main cellular constituent of blood, the shape and mechanical properties of RBC have a major impact on their in vivo flow behavior. Red cell aggregation is a normal physiological phenomenon that can affect flow dynamics and is the main determinant of low-shear blood viscosity. At stasis or at low shear rates, and in the presence of large macromolecules such as fibrinogen or dextran of appropriate molecular mass, erythrocytes attach loosely to each other in a characteristic face-to-face morphology, often referred to as rouleaux [3,4]. Such linear aggregates can also attach to each other to form complex nonlinear three-dimensional structures.

RBC aggregation is a reversible process and, under normal conditions, the attractive forces in such aggregates are relatively weak, with shear rates on the order of 20–40 s^{-1} sufficient to disperse them into individual cells [5]. Abnormal increases of RBC aggregation have been observed in several diseases associated with vascular complications [6–9], including diabetes, sepsis, hypertension and sickle cell anemia. An increased level of aggregation in pathological conditions can also have adverse effects on blood flow such as blunted velocity profiles [10], increasing flow resistance [11,12], greater axial migration of red cells and an expanding cell-poor layer near the vessel wall, a reduced red cell flux into vessel branches [2,12,13], decreasing functional capillary density [14,15], altered shear stress at vessel walls [16] and hence alterations of mechanisms controlling vessel dimensions [17].

Currently, there are two coexisting models postulated as the mechanism for RBC aggregation, the bridging model and the depletion model. In the bridging model, macromolecules adsorb onto the surface of two adjacent RBC and form so-called “bridges” between them. Aggregation occurs when the attractive forces due to adsorption exceed disaggregation forces due to electrostatic repulsion, membrane strain and shear forces [18–20]. This model seems similar to other cell–cell interactions such as agglutination, with the difference being that the attractive energy is much lower in the case of aggregation. The formal treatment of this mechanism has been done by Chien via a force-balance equation [21]. However, Brooks et al. have suggested that this model may not be appropriate for aggregation induced by proteins [22].

The depletion model, on the other hand, proposes an essentially opposite mechanism. In the depletion model, a lower polymer

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concentration near the cell surface relative to the bulk phase develops and hence the molecules are depleted from the surface and a depletion layer is formed resulting in an osmotic gradient between this depletion layer and the bulk phase. As two cells approach each other, their depletion layers begin to overlap expelling fluid from this intercellular gap, thereby generating an attractive force between the adjacent cells. According to this model, aggregation occurs when the attractive depletion force exceeds the abovementioned disaggregation forces. It should be noted that depletion interaction is a well-known phenomenon in colloidal chemistry and several reports regarding depletion flocculation can be found in the literature (e.g. [23–26]). However, less attention has been given to the applicability of this model to RBC aggregation [27–30].

The extent of RBC aggregation is known to be affected by both cellular (e.g., shape, glycocalyx structure) and suspending medium properties (e.g., protein concentration) [31]. This dependence has led to distinguishing between two terms: (1) aggregation, which is the extent of RBC aggregation; (2) aggregability, which refers to the intrinsic tendency of RBC to aggregate [32]. Even though it has been known for some time that both aggregation and aggregability are altered in several diseases [33], factors determining such alterations have not been identified. One reason for this might be that the specific mechanism inducing aggregation remains under discussion, thus making this identification a difficult if not impossible task. Hence, providing a theoretical framework that describes in detail the effects of different cellular and polymeric parameters on the aggregation is of paramount importance in order to gain a deeper insight of the pathophysiology of diseases associated with altered RBC aggregation. Consequently, the present study was designed to extend previous work [3,31,34] by employing a theoretical model based on the depletion mechanism [26] and considering glycocalyx structure and polymer interaction with the glycocalyx.

2. Theoretical considerations

To calculate the surface affinities between RBC in plasma-like suspensions, it is first necessary to define the nature of the cell–cell interaction. The RBC surface, termed its glycocalyx, consists of a layer of proteins and glycoproteins and bears a net negative charge that is primarily due to ionized sialic acid groups. In our previous reports [3,31,34], only depletion and electrostatic interactions were considered since due to the high electrostatic repulsion, the theoretical cell–cell distances at which a minimal interaction energy (i.e., maximal surface affinity) occur are always greater than twice the thickness of the cell's glycocalyx. Thus, steric interactions between glycocalices on adjacent RBC can be neglected; with van der Waals interactions' interaction energies in the order of a few $\mu J/m^2$, they are also omitted [35]. However, in these prior studies, the distribution of glycocalyx segments was assumed to be uniform, whereas this work considers the effects of the physical structure of the glycocalyx. Therefore, various potential segment distributions, as well as steric interactions, have to be considered in order to evaluate the effects of a more realistic structure and to obtain an improved understanding about possible drawbacks of previous simplifications.

2.1. Glycocalyx structure

In order to consider the interaction of the macromolecules in solution and the RBC surface it is also important to consider the surface structure. For this purpose, one has to consider the segment density as well as the segment distribution. The glycocalyx segment density is estimated by considering the overall carbohydrate mass of the cell membrane ($\sim 8.257 \times 10^{-14}$ g/cell) and assuming

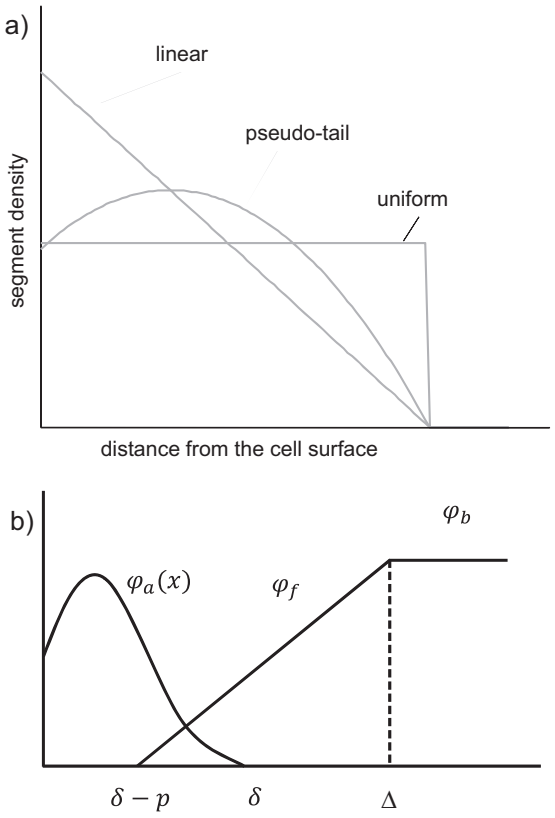

Fig. 1. (a) Schematics of the various segment distribution profiles; (b) concentration profiles of polymer and glycocalyx macromolecules.

that it consists of 7 kDa dextran chains [36]. The dextran chains are approximated as glucose segments, resulting in 1.97×10^{18} segments per square meter of the cell surface area. For the distribution of these segments, three different segment density profiles were chosen: (1) uniform; (2) linear; (3) pseudo-tail distribution. These distributions are schematically depicted in Fig. 1a [26] and can be mathematically described as indicated below:

- uniform distribution
$$\rho_a(x) = \bar{\rho}_a$$
- linear distribution
$$\rho_a(x) = 2\bar{\rho}_a \left(1 - \frac{x}{\delta}\right) \quad (1)$$
- pseudo-tail distribution
$$\rho_a(x) = \bar{\rho}_a \left(1 + \frac{2x}{\delta} - \frac{3x^2}{\delta^2}\right)$$

The subscript a indicates the attached layer (i.e., glycocalyx), $\bar{\rho}_a$ the average segment density, δ the glycocalyx thickness and the variable x stands for the distance from the cell surface.

2.2. Steric interaction

When two cells with attached macromolecules (i.e., glycocalyx) of thickness δ approach each other and the distance between them becomes less than twice the glycocalyx thickness, the attached macromolecules start to interpenetrate. Polymer segments of the adjacent surfaces interact with one another leading to an increasing energy of mixing [26]. If the distance decreases even further to less than the glycocalyx thickness, compression of the attached molecules will also occur, giving rise to an increase of the elastic free energy of the system. In order to evaluate steric interactions, the