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ARTICLE

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Interactions of erythrocytes with an artificial wall: influence of the electrical surface charge

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Abstract Electrical charge on any biological surface plays a crucial role in its interaction with other molecules or surfaces. Here, we study, under flow conditions, the interactions of erythrocytes with an artificial surface: a platinum microelectrode whose charge density ranges from –15 to +27 μC/cm^2. This artificial surface could be similar in surface charge to an endothelium or a biomaterial. In this model, interactions are measured as a transient relative increase of the electrolyte resistance obtained by impedance measurement of a microelectrode. A maximal interaction of erythrocytes with the charged surface is calculated in the 0 to +10 μC/cm^2 charge density range. At negative surface charge, a less efficient contact was obtained because of electrostatic repulsion forces. High positive surface charge (charge density >10 μC/cm^2) does not improve the contact but induces a progressive decrease in the contact efficiency, which could be explained by a rearrangement of macromolecules on the erythrocyte surface or an effect of positive groups on the cell membrane. This work suggests that a greater surface area of contact is obtained in the 0 to +10 μC/cm^2 charge density range and that this is provided by more molecular bridges.

Key words Surface charge · Charge density · Red cell · Artificial wall · Electrical impedance

1. Introduction

The distribution of electrical charge on any biological surface profoundly affects its interaction with any other surface or molecule. Changes in the electrical charge of blood cells or vessel walls may produce metabolic changes or alterations in blood flow properties, cell or vessel wall deformability, red cell aggregation, and adsorption of components from blood at any of the surfaces present.

Most biological surfaces exhibit a negative surface charge and, in the micovasculature, electrostatic repulsive forces exist between endothelial cells and erythrocytes which can account for the general property of non-adherence of circulating cells in normal blood vessels (Born and Palinski 1985; 1989). When sialic acid, located on glycoproteins in the membrane surface, is removed by enzymatic treatment of red cells with neuroaminidase, erythrocytes are trapped in selective regions and the degree of flow reduction can be correlated with the amount of neuraminidase-treated erythrocytes trapped (Simchon et al. 1988). This suggests that removal of neuraminidase-susceptible sialic acid, leads to a reduction of surface charges and plays a significant role in affecting the flow behaviour of erythrocytes in the circulation. Furthermore, erythrocytes undergo changes in their surface charge as a function of cell age and a ganglioside-linked sialic acid could be responsible for electrical surface charge differences between erythrocytes of different ages (Walter et al. 1986).

These results illustrate the importance of biological surface charge in the circulation and the role of sialic acid in the pathogenesis of vascular diseases. Our own interest arises from electrochemical studies on blood component interaction with a charged support. In particular, impedance measurements have been used to study protein adsorption at electrodes (Bernabeu and Caprani 1990; Caprani and Lacour 1991) and to examine the modification of an endothelial cell monolayer cultured on an electrode, following a chemical or shear stress stimuli (Tamisier et al. 1989). These studies show the complexity of the mechanisms involved. Interactions of red blood cells with a large electrode have also been studied, under sedimentation conditions, using glutaraldehyde-rigidified red cells (Gingell and Fornes 1976).

We therefore propose to investigate, under flow conditions, the effect of the charge density of an artificial wall on the interactions of erythrocytes with this support. The artificial wall is a microelectrode made of platinum. Negative surface charges simulated the negative charge of a nor-

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mal endothelium, a nearly neutral charge a treated endothelium, and positive charges a biomaterial positively charged. Impedance measurements were used to analyse electrochemically the erythrocyte/artificial wall interactions. The electrolyte resistance (R_e) determined by impedance measurement was the major parameter studies in this paper. The micro size of the electrode allowed us to follow individual interactions. The transient measurements of the impedance resulting from cell contact on the electrode in a flow chamber eliminated cellular changes associated with prolonged adhesion and allowed the rapid analysis of many cells.

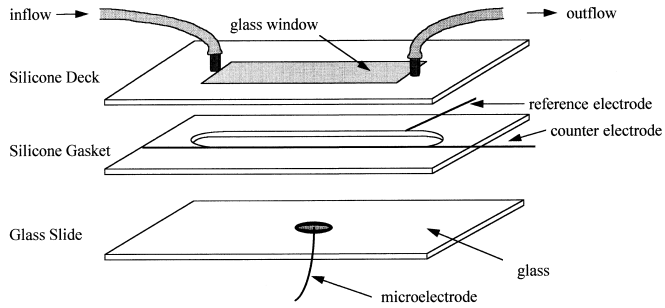

Fig. 1 The parallel plate flow chamber made of glass and silicone, including the three electrodes

2. Materials and methods

Preparation of human red blood cells

Fresh venous blood from normal, healthy donors was drawn into EDTA. Red blood cells were separated from whole blood by spontaneous erythrocyte sedimentation. Red cells were then suspended at the desired concentration in medium 199 (M199) (Sigma, France) supplemented with 10% foetal calf serum (JBio, France), 2 mM glutamine (Boehringer, Germany), 100 U/ml penicillin (Boehringer, Germany), 0.1 mg/ml streptomycin (Boehringer, Germany), 2.5 mg/ml B amphotericin (Boehringer, Germany), and 15 mM/ml Hepes (Gibco, France). Red blood cells were used within 5 hours after isolation.

Hydrodynamic model

To produce a well-defined flow, we used a parallel plate geometry flow chamber described in Fig. 1 (Godin et al. 1995). Briefly, the chamber consisted of three slides: (i) A glass slide with a small orifice which allowed the fixation of the working microelectrode made of platinum (Goodfellow, France). (ii) A 1 mm thick silicone gasket (Dow Corning, France) which defined the dimensions of the flow channel. The channel was 9 cm long and 0.5 cm wide. (iii) A silicone deck which presented a glass window for easy visual observations and allowed continuous feeding of the red cell suspension through the channel. The entire assembly was held together by an aluminium support. Under flow conditions, the channel Reynolds number was less than 5, indicating that flow was laminar. The wall shear rate was calculated by using the momentum balance for a Newtonian fluid, assuming a parallel-plate geometry and a fully developed flow. The wall shear rate (s^{-1}) is given by the formula:

$$\gamma = \frac{6Q}{a^2 b} \quad (1)$$

where Q is the volumetric flow rate (cm^3/s), a the channel height (cm), and b the channel width (cm). A constant and regular flow in the channel was delivered by a pump with a magnetic drive (Asservissement Electronique, Bagnolet, France). Microscopic observations and videotaped images were periodically used to confirm individual interactions of erythrocytes with the working electrode.

Impedance measurements

The interactions between erythrocytes and the working electrode were analysed by measuring the electrical impedance (Godin et al. 1995). A three-electrode potentiostatic device (Potentiostat Tacussel PJT 16-0.6, Solartron, France) was used. The working microelectrode was a 20 μm diameter platinum electrode (3.1×10^{-4} mm^2), mirror-polished with alumina abrasives (Presi, France). Various sizes of alumina particles were used, the smallest being one μm. The reference electrode consisted of a section of a 300 μm diameter platinum wire (7.1×10^{-2} mm^2) whose potential was in equilibrium with the medium. It was inserted into the silicone gasket perpendicularly to the flow. The potentiostat controlled voltage between this electrode and the working electrode, and consequently maintained the working electrode at a given surface charge. The counter electrode consisted of a platinum wire (Goodfellow, France) disposed along on the length of the channel and providing a large surface area (85 mm^2) in comparison with the working electrode surface area. Thus, the ohmic drop between these two electrodes was located in the immediate vicinity of the working electrode.

Differential impedance was performed through a 1250 Schlumberger Frequency Response Analyser (Solartron, France). The amplitude of the sine wave potential perturbation was 10 mV, a signal of sufficiently weak amplitude providing a linear response of the system. Moreover, with such a signal level there was no local heating because there is an electrical power lower than 1 nW according to the values of the impedance. Concerning the steady state current, which varied from 1 pA/cm^2 to a few $\mu A/cm^2$ with the imposed potential, the electrical power remained lower than 1 μW. Such electrical power values were not able to increase the temperature of the red cells, which remained for only a few seconds on the microelectrode.

Measurements were performed in the potential range from –750 and +750 mV/platinum reference electrode. Considering that the potential of the reference electrode is

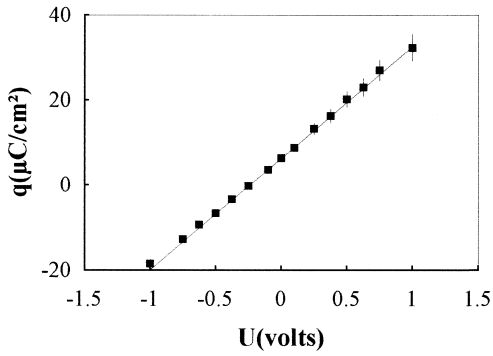

Fig. 2 Variation of the surface charge density of the working microelectrode with the potential (U/platinum) of the microelectrode. Microelectrode diameter = 20 μm. f = 1000 Hz. $\gamma = 140\ s^{-1}$. ■: experimental data. –: linear regression. The data are means ± standard deviation SD (vertical bars) from four experiments

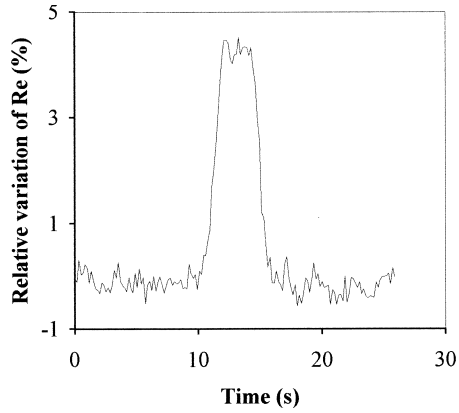

Fig. 3 Example of the electrical response due to the contact of an erythrocyte with the working microelectrode. f = 50 kHz. $q = 6.3\ \mu C/cm^2$. $\gamma = 11 \pm 1\ s^{-1}$

+220 mV/Saturated Calomel Reference (SCE) in medium M119 (Pech et al. 1993) and that the zero charge potential is –20 mV/SCE for the platinum (Baszkin and Lyman 1980), the zero charge potential is –240 mV/platinum reference electrode. Then, the charge density $q(\mu C/cm^2)$ is equal to:

$$q = \int_{-0.240}^{U} C_d \, dU \qquad (2)$$

where U (V) is the potential/platinum of the microelectrode and $C_d(\mu F/cm^2)$ the double-layer capacitance. C_d is calculated from the imaginary part of the impedance (see below). Figure 2 shows that q was linear in the range of potentials studied.

According to previous studies (Caprani and Lacour 1991; Godin et al. 1995; Caprani and Nakache 1983; Gabrielli 1984), it is assumed that the equivalent electrical network of the electrolyte/electrode interface consists of an electrolyte resistance R_e in series with a circuit made of the double-layer capacitance C_d in parallel with the charge transfer resistance R_t, within the frequency range studied. Thus, the impedance Z is given by:

$$Z = R_e + \frac{R_t}{1 + R_t^2 C_d^2 \omega^2} - j \frac{R_t^2 C_d \omega}{1 + R_t^2 C_d^2 \omega^2} = a + jb \qquad (3)$$

whee ω is the angular frequency and a and b are the real and imaginary parts of the impedance, respectively. A high frequencies, the real part of the impedance is assimilated into the electrolyte resistance R_e. In our study, only the time variation of R_e has been described (at 50 kHz). The results were processed using an IBM compatible computer (Kenitec, 486DX33, France).

Assuming that red cells behave as insulating particles, the electrical resistance of an erythrocyte suspension has been shown to be a function of the fraction of total volume occupied by the suspended phase, ρ (Velick and Gorin 1940). For our experimental conditions, ρ remained below 0.0001, and the suspension resistance can be assimilated into the medium (electrolyte) resistance. In consequence, the suspension resistance was assumed to be independent of the volume occupied by erythrocytes in the suspension, and variations of the electrolyte resistance (R_e) were only due to the microelectrode surface area decrease which occurred when an erythrocyte was in contact with the microelectrode. Furthermore, as R_e is inversely proportional to the radius of the electrode (Bard and Faulkner 1980), a blood cell contact resulted in a decrease of the effective radius of the microelectrode and consequently in an increase of R_e. Figure 3 shows an example of a red cell contact with the microelectrode corresponding to a transient increase of R_e. A relative increase of R_e is related to a surface reduction (ΔS) which may be expressed as:

$$\Delta S = \pi R_0^2 \frac{\alpha(2+\alpha)}{(1+\alpha)^2} \qquad (4)$$

where R_0 is the microelectrode radius and α the relative increase of R_e. The surface reduction, ΔS, is equivalent to the surface area of one erythrocyte whose contact diameter was as follows:

$$\text{contact diameter} = 2R_0 \frac{\sqrt{\alpha(2+\alpha)}}{1+\alpha} \qquad (5)$$

There is probably a current flow between the microelectrode and the glycocalix of red cells, which could lead to an effective electrode surface area slightly smaller than the above ΔS calculated from Eq. (4). However, to simplify the model, this effect was neglected in the present study. This simplification was justified by the validity of the results presented below.

3. Results

a. Effect of charge density on the contact efficiency

Charge densities ranging from –15 to +27 $\mu C/cm^2$ have been investigated. Red cells were resuspended in medium

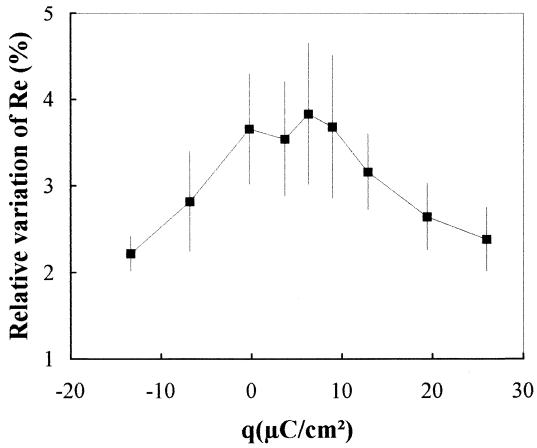

Fig. 4 Contact efficiency of erythrocytes with the microelectrode. Relative variation of the electrolyte resistance with the charge density of the working microelectrode at low wall shear rate: $\gamma = 11 \pm 1\ s^{-1}$. f = 50 kHz. $C = 10^6$ cells/ml. Data points are means and verticals bar represent SD ($p < 0.01$)

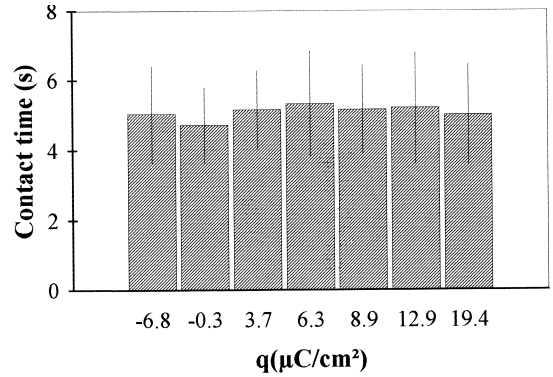

Fig. 5 Contact time of erythrocytes with the microelectrode for different donors at various surface charge densities of the microelectrode. $\gamma = 11 \pm 1\ s^{-1}$. f = 50 kHz. $C = 10^6$ cells/ml. Data points are means and vertical bars are SD

M199 at a concentration of 10^6 cells/ml, which was a sufficiently low concentration to analyse individual interactions. For all experiments, whall shear rate was maintained at a low value (i.e. $11 \pm 1\ s^{-1}$) to allow erythrocytes to move slightly on the microelectrode for a few seconds and to analyse slow events. In the medium red cell velocity was much more important. For each donor (n = 5) and at each charge density, impedance measurements were recorded for 16 to 20 minutes, which allowed a sufficient number of individual transient interactions for statistical calculations.

Figure 4 shows the mean relative increase of R_e as a function of charge density. A maximum was obtained in the 0 to +10 μC/cm^2 charge density range, with an average of $3.8 \pm 0.8\%$ maximal variation of R_e. This indicated that the efficiency of contact was maximal in this range and, according to Eq. (5), the contact diameter of one erythrocyte was 5.4 ± 0.5 μm. When the charge density of the microelectrode was negative or above +10 μC/cm^2, the relative variation of R_e progressively decreased to about $2.2 \pm 0.2\%$, corresponding to a 42% decrease of the maximal value and a reduction of the contact diameter of 4.1 ± 0.2 μm.

b. Effect of charge density on contact time

Experimental procedures were the same as above: the red cell concentration was 10^6 cells/ml and the wall shear rate was $11 \pm 1 s^{-1}$. The charge density ranged from –7 to +20 μC/cm^2. The number of donors was 5. At each charge density, the impedance was measured for 16 to 20 minutes, providing numerous individual interactions of red cells with the microelectrode. From Fig. 5, the contact time did not appear to be dependent on the charge density (according to the standard deviation, there was no significant difference). In consequence, in the charge density range studied, a mean contact time of 5.1 ± 1.3 s was calculated.

4. Discussion

The attractive force between an erythrocyte and a biological or artificial material is provided by adsorption forces of macromolecules onto the cell surface. These forces include electrostatic attraction/repulsion, hydrogen bonding and Van der Waals forces, in addition to specific forces on biological material. The medium M199 has a conductivity of 0.015 $\Omega^{-1} m^{-1}$ (data not shown) and according to the study of Engelhardt et al. (1988), at the frequency used (50 kHz), Maxwell-Wagner effects can be neglected, and the adhesion of erythrocytes on the microelectrode was not therefore thought to be determined by a force generated by Maxwell-Wagner polarization of the cells.

Red blood cells are known to be negatively charged, and it is generally accepted that sialic accounts for a major portion of this negative surface charge (Eylar et al. 1962; Seaman and Uhlenbruck 1963; Chien 1975). Endothelial cells (Danon and Skutelsky 1976) are also negatively charged. Removal of sialic acid negative charges from the endothelium increases flow resistance (Born and Palinski 1989), demonstrating that blood flow through the microcirculation is facilitated by electrostatic repulsion between circulating cells and capillary walls. Changes in this balance could induce crucial microcirculatory disorders. The treatment of biomaterials is also of importance: for example, a positively charged biomaterial (a hydrophilic copolymer ethylene diamine-acrylic acid-acrylamide-estane) adsorbs higher amounts of fibronectin, von Willebrand factor and fibrinogen than materials negatively charged. In consequence, the adhesion of platelets was greater on the posi-

tively charged material than on any other materials (Sapatnekar et al. 1995). The release of substances by activated platelets can then promote cellular adhesion (Morley and Feuerstein 1989).

The present study has shown that changes in electrical surface charge of the wall can alter the contact efficiency of erythrocytes. Using electrophoretic mobility studies, some authors (Vargas et al. 1989) have calculated the charge density of endothelial cells from bovine artery, i.e. –8.7 $\mu C/cm^2$. One can assume that, in our model, the studied range of charge density fitted the normal behaviour of the endothelium.

When the microelectrode was negatively charged, electrostatic repulsion forces reduced the contact, but contact still occurred as the aggregation of two negatively charged red cells is possible (Chien 1975). Erythrocytes were probably adsorbed by Van der Waals forces or hydrogen bonding. Electrostatic repulsion reduced the contact efficiency to a $+2.2 \pm 0.2\%$ R_e variation, whereas an average maximal R_e variation of $+3.8 \pm 0.8\%$ was obtained for positive charge (from 0 to 10 $\mu C/cm^2$). This resulted in a 42% R_e variation reduction and a 24% reduction of the contact diameter.

A moderate positive charge of the microelectrode (0 to +10 $\mu C/cm^2$) contributed to a maximal contact efficiency ($+3.8 \pm 0.8\%$) and red cells were probably adsorbed by electrostatic attraction between the negative charge groups of red cells and the positively charged electrode surface. When the electrical surface charge of the microelectrode was above +10 $\mu C/cm^2$, a decrease in the R_e variation was observed until reaching 47% of the maximal value obtained in the 0 to +10 $\mu C/cm^2$ range. It is well known that the ionic strength of the suspending medium is a crucial factor which modulates the electrostatic repulsive force between two charged surfaces, for instance erythrocytes (Chien 1975). However, one cannot assume a change of the ionic strength of the medium (which remained the same for all experiments) even in the immediate vicinity of the surface. Indeed, in the potential range studied, no significant electrolytic reaction occurred (values of steady state current were less than a few $\mu A/cm^2$) and erythrocyte concentration was low, resulting in very slow consumption of nutrients during the experiments. It is possible that at high positive surface charge the conformation of the glycoproteins at the red cell surface might change and lead to a reduction of contact. Another possible explanation is that serum albumin, which presents both neutral and negative subunits, could be adsorbed in multiple layers on the microelectrode (Lacour et al. 1993) and this could lead to an apparent negative surface charge of the microelectrode. A final tenable explanation is that cationic charge groups at the erythrocyte membrane surface exert an effect at high positive surface charge of the support. If an attractive force occurred between negative groups of the erythrocyte membrane and the positive surface charge of the microelectrode, a repulsive force could appear between positive groups of the membrane and the positively charged microelectrode.

Contact time appeared to be surface charge independent as red cells were not stopped or slowed down by attractive forces. Furthermore, microscopic observations and videotape recordings at various charge densities (data not shown) did not induce erythrocyte shape changes: erythrocytes remained biconcave. Thus, shape changes could not be responsible for the reduced contact diameter. In consequence, it could be presumed that red cells were closer to the microelectrode in the 0 to +10 $\mu C/cm^2$ range of charge density studied, resulting in a more efficient contact. This hypothesis was supported by the fact that the contact diameter was larger when charge densities ranged from 0 to +10 $\mu C/cm^2$. As demonstrated in Fig. 6, a strong attractive force could press the erythrocyte onto the microelectrode surface; the deformability of the red cell membrane facilitates the formation of multiple macromolecular bridges (Chien 1975) and consequently should increase the total surface area of red cells in contact with the artificial wall.

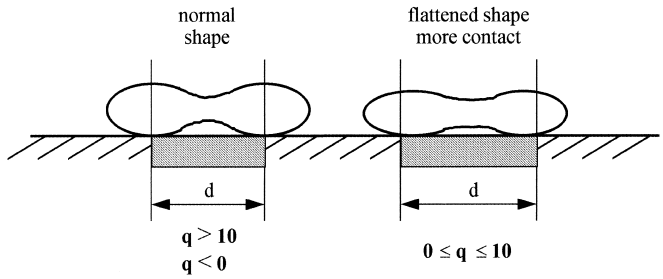

Fig. 6 Schematic representation of the contact of one erythrocyte following the surface charge density ($\mu C/cm^2$) of the microelectrode. *d* = contact diameter

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