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ARTICLE

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Effect of blood storage on erythrocyte/wall interactions: implications for surface charge and rigidity

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Abstract In this report, we study, under flow conditions, the interactions of stored erythrocytes with an artificial surface: a microelectrode whose charge density ranges from –15 to +27 μC/cm^2. Interactions consist of red cells slowly circulating on the microelectrode and exerting a real contact with the electrode. Interaction is detected and measured by transient fluctuations of the electrolyte resistance obtained by impedance measurement of the microelectrode. Effects of aging induced by storage of whole blood at 4 °C show that the surface charge of erythrocytes rapidly decreases when blood is stored for more than 6 days under our experimental conditions. In comparison with trypsin-treated erythrocytes, an eight day storage induces a 60% decrease in the surface charge of red cells. After two weeks of storage, red cells are no longer negatively charged, presumably be cause of removal of sialic acid. Cells rigidity is significant after 6 days of storage and influences the electrical contact. Membrane rigidity increase could arise from the surface charge decrease. Finally, the surface charge decrease could be of importance in the use of stored blood.

Key words Red cell · Aging · Charge density · Deformability · Electrical impedance

1 Introduction

Changes in the electrical charge of blood cells may produce alterations in blood flow properties, cell deformability, red cell aggregation, and adhesion of circulating cells at vessel walls. In particular, erythrocytes, like most biological surfaces, exhibit a negative surface charge that is mainly attributed to sialic acid residues located on glycoproteins in the membrane surface (Eylar et al. 1962; Seaman and Uhlenbruck 1963). The electrostatic repulsion between red blood cells reduces erythrocyte aggregation. In diabetic patients, a decrease in sialic acid content has been observed and is associated with increased erythrocyte aggregation (Rogers et al. 1992). Furthermore, neuraminidase and trypsin treatment of red cells increases erythrocyte aggregation by removing sialic acid from the red cell surface. After this treatment, even at high shear rate, dispersion of erythrocyte aggregates remains incomplete (Böhler et al. 1993).

In the microvessels, electrostatic repulsive forces between erythrocytes and endothelial cells facilitate blood flow because of the non-adherence of circulating cells in normal blood vessels (Born and Palinski 1985, 1989). When sialic acid is removed by enzymatic treatment of red cells with neuraminidase, erythrocytes are trapped in particular regions and a flow reduction is induced (Simchon et al. 1988). These results demonstrate that a reduction of surface charges plays a significant role in affecting the flow behaviour of erythrocytes in the microcirculation, and illustrate the importance of sialic acid in the pathogenesis of vascular diseases.

Moreover, during storage at 4 °C, red blood cells undergo biochemical and physicochemical modifications. In blood banks, red cells are generally stored for 35 to 42 days at 4 °C with an acceptable 24 h post-transfusion viability (Hogman et al. 1985; Simon et al. 1987; Carmen et al. 1988), although red cell storage in some treated-polyvinylchloride bags should be limited to 21 days (Myhre et al. 1987). In fact, important deteriorations in red cell morphology and in deformability were observed during storage (Hogman et al. 1985), and morphological changes are generally associated with a reduced deformability of the erythrocyte (Chabanel et al. 1987). Red cell viability is generally evaluated by measurements of pH, hemolysis, adenosine triphosphate levels, glucose levels, etc. (Habibi et al. 1985; Simon et al. 1987), and these measurements do not take into account the surface charge decrease of the erythrocyte membrane. For example, a

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rapid decrease in the electrophoretic mobility has been observed during the first ten days of storage (Letterier et al. 1979) and this can be related to a surface charge reduction. Even in vivo, erythrocytes undergo systematic changes in their surface charge-associated properties as a function of cell age (Walter 1985) and older cells are less negatively charged than younger ones.

Furthermore, blood component interactions with a charged surface have already been studied by electrochemical methods. In particular, impedance measurement was used to determine the kinetics of adsorption of small organic molecules or proteins at electrodes (Bernabeu and Caprani 1990; Caprani and Lacour 1991) and to analyse the modification of an endothelial cell monolayer cultured on an electrode, following chemical or shear stress stimuli (Tamisier et al. 1989). More recently, this method was used to examine the contact of erythrocytes with a charged microelectrode (Godin et al. 1995; Godin and Caprani 1996). We therefore propose to investigate, under flow conditions, the effect of whole blood storage at 4 °C on the erythrocyte/wall interactions. A platinum microelectrode stimulated the charged wall and the electrolyte resistance (R_e), determined by impedance measurement, was the major parameter studied in this paper. Individual interactions of red cells with a microelectrode were analysed. Contact modifications of red cells circulating on the microelectrode for a few seconds are discussed, including the alterations of the surface charge and deformability of the red cell membrane.

2 Materials and methods

Preparation of human red blood cells

Fresh venous blood from normal, healthy donors was drawn into EDTA. Whole blood was stored at 4 °C in Vacutainer, (Polylabo, France). Red blood cells were separated from whole blood by spontaneous erythrocyte sedimentation. Red cells were 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.

In some experiments, sedimented cells (0.5 ml) were resuspended in 5 ml PBS without Ca^{2+} and Mg^{2+} (Gibco, France) containing 75 U or 150 U trypsin (Boehringer, Germany). The erythrocyte suspension was incubated at 37 °C for 30 min, then washed and resuspended at the desired concentration in M199 supplemented as above.

Experimental apparatus

The in vitro fluid shear stress in the microvasculature was simulated using a parallel plate geometry flow chamber as described in Fig. 1 (Godin et al 1995; Godin and Caprani 1996). Briefly, the chamber consisted of a glass slide which allows the insertion of a working microelectrode made of platinum (Goodfellow, France), a 0.1 cm thick silicone gasket (Dow Corning, France) wich defined the dimensions of the flow channel (9×0.5 cm), and the silicone deck which provided 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 (g, s^{-1}) was calculated as follows:

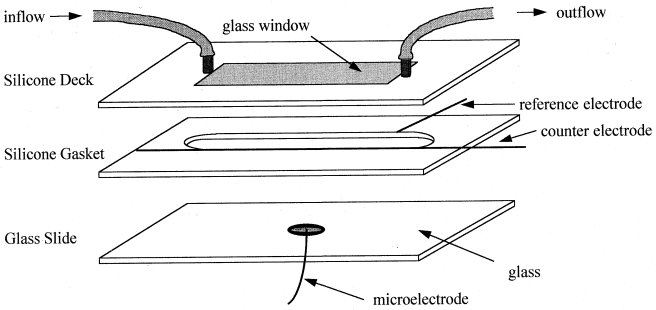

Fig. 1 Description of the parallel plate flow chamber made of glass and silicone

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

where Q was the volumetric flow rate (cm^3/s), a the channel height (0.1 cm), and b the channel width (0.5 cm). A constant flow in the channel was delivered by a pump with a magnetic drive (Asservissement Electronique, Bagnolet, France).

Impedance measurements

The interactions between erythrocytes and the working microelectrode were analysed by measuring the electrical impedance. A three-electrode potentiostatic device (Potentiostat Tacussel PJT 16-0.6, Solartron, France) was used. The working microelectrode consisted of 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 until reaching 1 µm in size. The reference electrode was the 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 perpendicularly to the flow. The potentiostat controlled voltage between this electrode and the working electrode, and maintained the working electrode at a given surface charge. The counter electrode consisted of a platinum wire (Goodfellow, France) and provided a large surface area (85 mm^2) in comparison with the working microelectrode surface

area, so that 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 to provide a linear response of the system. The applied potential ranged from –750 to +750 mV/platinum reference electrode. Considering that the potential of the reference electrode is +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 ($\mu C/cm^2$) was as follows:

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

where U (V) was 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). The charge density q was linear in the range of potential studied (Godin and Caprani 1996).

According to previous studies (Caprani and Nakache 1983; Caprani and Lacour 1991; Gabrielli 1984), it is assumed that, within the frequency range studied, 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. Thus, the impedance Z was:

$$Z = R_e + \frac{R_t}{1 + R_t^2 C_d^2 \omega^2} - j\frac{R_t^2 C_d \omega}{1 + R_t^2 C_d^2 \omega^2} \tag{3}$$

where w was the angular frequency. At high frequencies, the real part of the impedance was assimilated to the electrolyte resistance R_e. In the present study, only R_e variation 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 the erythrocyte suspension is a function of the fraction of total volume occupied by the suspended phase, r (Velick and Gorin 1940). Under our experimental conditions, r remained below 0.0001, the suspension resistance can thus be assimilated into the medium (electrolyte) resistance. The suspension resistance was assumed to be independent of the volume occupied by the erythrocytes in the suspension, and R_e variations were only dependent on 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 (R) of the microelectrode and a transient increase of R_e (see an example of the electrical signal of an erythrocyte contact with the microelectrode in Fig. 2).

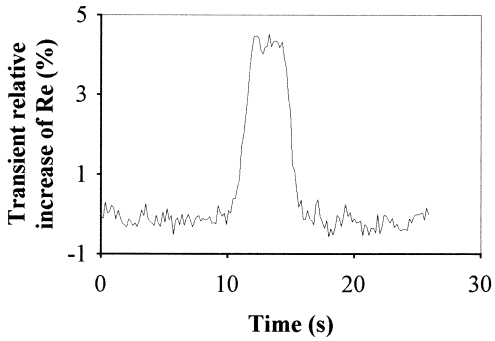

Fig. 2 Example of the electrical signal due to the contact of an erythrocyte with the working microelectrode. f = 50 kHz, g = 11 ± 1 s^{-1}

Then, the relative increase, of R_e (a) was:

$$\alpha = \frac{R_0 - R}{R} \tag{4}$$

where R_0 was the microelectrode radius. A surface reduction (DS), due to the contact of one erythrocyte with the microelectrode, was defined as $\Delta S = \pi R_0^2 - \pi R^2$. From Eq. (4):

$$\Delta S = \pi R_0^2 \frac{\alpha(2+\alpha)}{(1+\alpha)^2} \tag{5}$$

The surface reduction, DS, was equivalent to the surface area of one erythrocyte and can also be expressed as $\Delta S = \frac{\pi}{4}$ (contact diameter)^2; then, contact diameter was as follows:

$$\text{contact diameter} = 2R_0 \frac{\sqrt{\alpha(2+\alpha)}}{1+\alpha} \tag{6}$$

The contact time was measured from the width of the resistance increase. An eventual current flow between the microelectrode and the glycocalix of red cells has been neglected to simplify the model.

3 Results

3.1 Contact efficiency as a function of blood storage duration

The influence of whole blood storage at 4 °C on the contact efficiency of red cells has been investigated with the microelectrode maintained at various surface charge densities (Fig. 3). For clarity, only data means are represented in the figure and whole results with standard deviation (SD) are displayed in Table 1. Charge density ranged from –15 to +27 $\mu C/cm^2$, wall shear rate was maintained at 11.0 ± 0.6 s^{-1} to analyse slow events, and the erythrocyte concentration was 10^6 cells/ml to allow individual contacts. Erythrocytes from whole blood stored for up to 14 days at 4 °C were used. A maximal transient relative increase of R_e was observed in the [0, +10 $\mu C/cm^2$] range of charge densities, corresponding to a maximal contact efficiency. This has already been observed in a previous study

Table 1 The relative increase of the electrolyte resistance. Effect of the microelectrode charge density on erythrocyte contact. Data are means ±SD (n≥5)

q (μC/cm²)	Storage duration				
	Fresh blood	3 days old	6 days old	10 days old	14 days old
–13.38	2.09±0.42	2.00±0.41	1.86±0.41	1.03±0.09	–
–6.82	2.54±0.44	2.47±0.39	2.19±0.41	1.18±0.31	0.87±0.14
–0.26	3.20±0.49	2.78±0.30	2.48±0.47	1.57±0.17	0.99±0.16
3.67	3.34±0.47	3.25±0.45	2.47±0.27	1.32±0.28	1.08±0.20
6.30	3.10±0.22	3.01±0.43	2.27±0.33	1.27±0.13	1.12±0.22
8.92	3.26±0.48	3.22±0.28	2.56±0.36	1.38±0.22	1.14±0.19
12.85	2.87±0.40	2.57±0.42	2.19±0.41	1.24±0.23	1.05±0.16
19.41	2.46±0.38	2.21±0.37	2.11±0.42	0.99±0.06	0.89±0.12
25.97	2.09±0.32	2.21±0.31	1.85±0.27	0.91±0.17	–

Table 2 Contact time of erythrocytes with the microelectrode. Blood aging was induced by storage at 4°C. Data were means ±SD (n≥5)

q (μC/cm²)	Contact time (sec)				
	Fresh blood	3 days old	6 days old	10 days old	14 days old
–6.82	2.7±0.4	3.3±0.4	3.4±0.6	2.7±0.8	1.3±0.4
–0.26	3.4±0.5	3.8±0.5	3.1±0.6	3.0±0.5	2.0±0.3
3.67	3.6±0.6	3.6±0.5	3.0±0.4	3.1±0.5	2.0±0.4
6.30	3.9±0.6	3.7±0.8	3.6±0.6	3.1±0.6	1.9±0.3
8.92	3.8±0.6	3.8±0.6	3.3±0.5	2.9±0.6	1.9±0.4
12.85	3.0±0.6	3.1±0.5	3.2±0.3	2.4±0.6	1.5±0.4
19.41	3.1±0.5	3.5±0.6	3.2±0.5	2.6±0.3	1.3±0.3

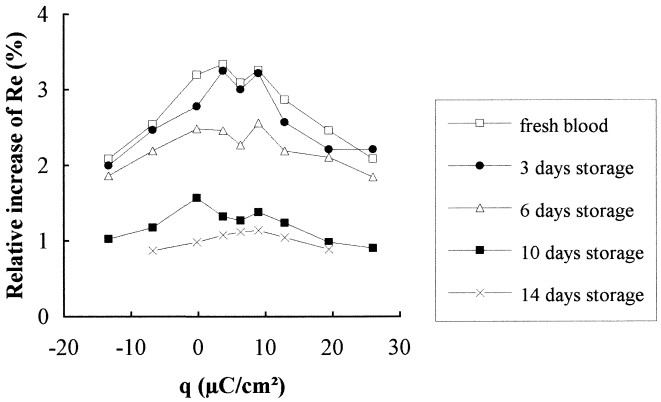

Fig. 3 Effect of storage on the contact efficiency of erythrocytes. Variation of the relative increase of the electrolyte resistance with the surface charge of the working microelectrode. g = 1.0 ± 0.6 s^{-1}, f = 50 kHz, C = 10^6 cells/ml. Data points are means with standard deviation SD (n≥5)

and thoroughly described (Godin and Caprani 1996). The maximal transient R_e increase progressively decreased with storage duration, resulting in a reduced contact efficiency.

Contact of fresh erythrocytes with the microelectrode contributed to a maximal transient R_e increase of +3.24±0.45%. After 3 days of storage, the maximum remained at the same value (+3.07±0.42%), decreased to 2.46±0.36% at 6 days of storage, whereas only a +1.40±0.19% maximal R_e increase was calculated after 10 days of storage. Until 6 days, no significant decrease in the contact efficiency was noticed at negative charge densities (<–6 μC/cm²) and at high positive charge densities (>+19 μC/cm²) of the microelectrode. No significant difference of transient R_e increase was observed with fourteen-day-old red blood cells at various charge densities, and the transient increase of R_e remained at +1.0±0.2% whatever the microelectrode charge density.

3.2 Contact time was a function of blood storage

Wall shear rate was maintained at 11.0±0.6 s^{-1} and the red cell concentration was 10^6 cells/ml. Contact time was defined here as the passing time of one erythrocyte slowly circulating on the microelectrode, which exhibited a real contact with the electrode. A slight decrease of contact time was observed when the charge density was less than –6 μC/cm² or more than +13 μC/cm² (see Table 2) in comparison with the contact time obtained in the range [0, +10 μC/cm²]. This was apparent for fresh or 3 day old blood. However, the most important result was the contact time decrease with aging. Red cells from fresh whole blood showed a contact time of about 3.7 seconds and the contact time remained the same for the first 6 days of storage. After that, contact time rapidly decreased and was reduced to 1.9 seconds at 14 days of storage.

3.3 Effect of trypsin treatment on red cell contact

Trypsin has been used instead of neuraminidase which is known to remove all the sialic acid present at the cell surface. To compare the effect of trypsin with our experimental results an incomplete removal of sialic acid had to be obtained in order to estimate our charge changes.

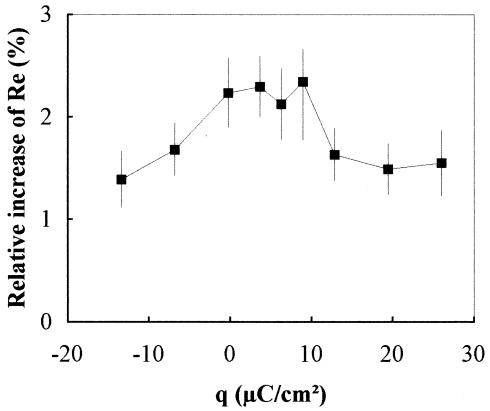

Fig. 4 Trypsin-treated erythrocytes. Relative increase of the electrolyte resistance at various charge densities of the microelectrode. $g = 10.7 \pm 0.4\ s^{-1}$, f = 50 kHz, $C = 10^6$ cells/ml. Data points are means with SD ($n \geq 8$)

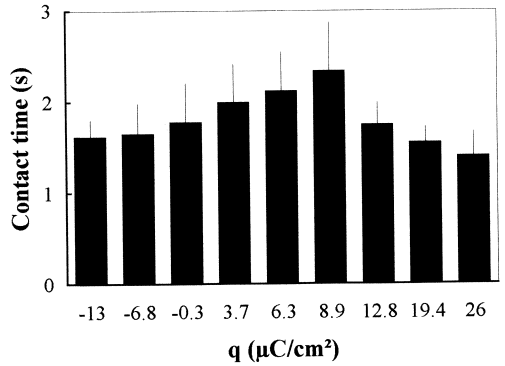

Fig. 5 Contact time of trypsin-treated erythrocytes with the microelectrode at various charge densities. $g = 10.7 \pm 0.4\ s^{-1}$, f = 50 kHz, $C = 10^6$ cells/ml. Data points are means with SD ($n \geq 8$)

Table 3 Effect of trypsin concentration on the relative increase of R_e expressed as the percentage of control value (%) at various charge densities of the microelectrode. Data were means ±SD ($n \geq 3$)

q (µC/cm²)	Trypsin concentration	
	75 U	150 U
–6.8	63.7 ± 13.0	66.2 ± 11.6
–0.3	62.0 ± 9.2	69.9 ± 10.5
+8.9	72.0 ± 5.1	71.8 ± 11.6

First, two trypsin concentrations were tested, 75 U and 150 U per 0.5 ml sedimented erythrocytes in 5.5 ml total volume. At both concentrations and for various charge densities of microelectrode, the transient relative increase of R_e was compared to the control (Table 3). When red cells were treated with 75 U trypsin, the transient relative increase of R_e was only 62–72% of the control value. A similar effect was produced with 150 U trypsin and results obtained at various charge densities of the microelectrode did not exhibit a significant difference. These findings suggested that a trypsin concentration of 150 U per 0.5 ml sedimented erythrocytes contributed to a maximal effect and can be assimilated to an optimal concentration.

Using this concentration, the influence of the charge density of the microelectrode has been examined on trypsin-treated erythrocytes from fresh whole blood (Fig. 4). Wall shear rate and erythrocyte concentration were maintained at $10.7 \pm 0.4\ s^{-1}$ and 10^6 cells/ml. A maximal transient R_e increase was observed from 0 to +10 µC/cm² with a mean value of 2.2%. Negative and high positive charge densities (>10 µC/cm²) resulted in less efficient contact, transient R_e increase was reduced by about 35%. Furthermore, contact time appeared to be charge density dependent (Fig. 5). Contact remained for 2.3 seconds at 8.9 µC/cm², progressively decreased to 1.7 seconds at negative charge densities (<–7 µC/cm²), and to 1.6 seconds at high positive charge densities (>+13 µC/cm²).

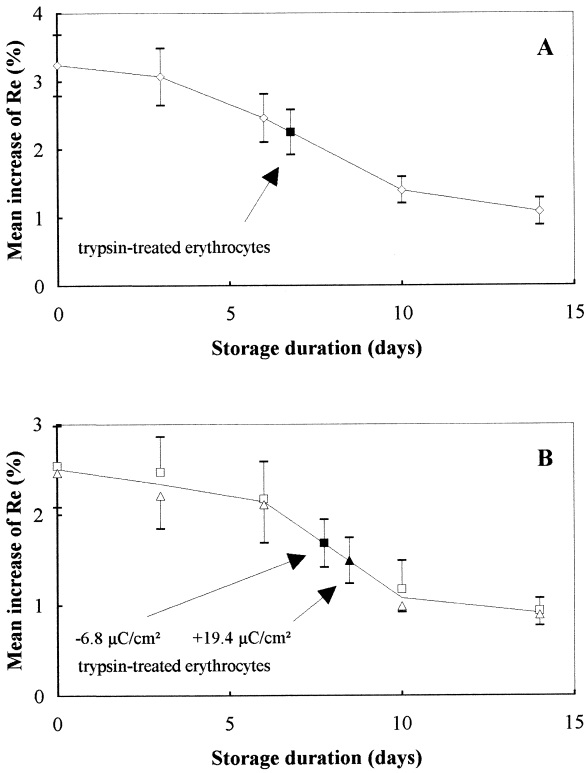

Fig. 6A, B Correlation between stored red blood cells and trypsin-treated red cells. **A** mean value calculated in the range [0, +10 µC/cm²]. **B** ○: values at negative (–6.8 µC/cm²). Δ: values at positive (+19.4 µC/cm²) charge density of the microelectrode. Mean data ±SD

3.4 Correlation between red cells from stored blood and those treated with trypsin

To estimate the surface charge reduction of red cell with in vitro aging, the transient R_e increase of stored blood and trypsin-treated red cells were compared (Fig. 6a, b). For each storage period, a mean value of transient relative R_e increase was calculated from Table 1 in the [0, +10 µC/cm²] charge density range and the transient increase of R_e

obtained with trypsin-treated erythrocytes was equivalent to 7 days of storage (Fig. 6a). At negative (–6.8 μC/cm^2) and positive (+19.4 μC/cm^2) charge densities, the same curve of transient increase of R_e with storage duration was determinated (Fig. 6b) and the R_e increase of trypsin-treated erythrocytes was comparable to 8 days of storage.

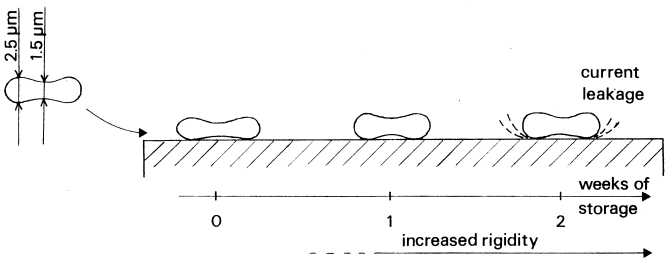

Fig. 7 Schematic illustration of the contact of one red cell with the microelectrode at various storage durations. During the first few days of storage, cells are easily deformable and produce a larger contact surface area than at one week of storage. At two weeks, contact is not perfectly isolating, because of the increased rigidity of cells

4 Discussion

Forces between a red blood cell and a biological or an artificial wall include electrostatic attraction/repulsion, hydrogen bonding and Van der Waals forces. Under normal circumstances, biological surfaces are negatively charged. In particular, erythrocytes have a negative electrical surface charge, and sialic acid accounts for an important part of this negative charge (Eylar 1962; Seaman and Uhlenbruck 1963; Chien 1975). Endothelial cells are also negatively charged (Danon and Skutelsky 1976), and removal of sialic acid negative charges from the endothelium increases flow resistance (Born and Palinski 1989), suggesting that electrostatic repulsion between blood cells and capillary walls facilitates blood flow through the microvasculature.

Other studies have observed that conservation of red cells in the blood bank alters the surface charge of erythrocytes and increases the filtration time (Nakache et al. 1983) or decreases the cell deformability and the electrophoretic mobility (Leterrier et al. 1979). Normal and fresh red cells are easily deformable and recover their shape immediately on removal of the deforming force. Red cell deformation is necessary to allow passage through capillaries whose diameter is less than the cell dimensions and a reduction in red cell deformability causes preferential trapping of the rigidified red cells in selected organs and tissues (Chien 1992).

Normal and fresh erythrocytes have an electrical surface density of about –3 μC/cm^2 (Vargas et al. 1989; Van Damme et al. 1994). In the present study, we have simulated a rapid and significant change in the electrical surface charge of the erythrocyte membrane by storage of whole blood at 4 °C. Various surface charge densities of the support have been examined to simulate a normal negatively charged endothelium, an endothelium treated to remove negative surface charge and biomaterials negatively or positively charged. As previously explained (Godin and Caprani 1996), under our experimental conditions, adhesion of erythrocytes on the microelectrode was assumed to be independent of any force generated by Maxwell-Wagner polarization of the cells (see Engelhardt et al. 1988).

During the first few days of storage (3–6 days) no important changes in the surface charge and rigidity of red cells were observed: contact efficiency and time contact remained about the same whatever the charge density. A maximal contact efficiency was obtained when charge density was positive and ranged from 0 to +10 μC/cm^2, as explained before. Briefly, attraction occurred between red cells and the positive surface, leading to a maximal surface area of contact, and consequently to a maximal reduction of the microelectrode surface area, resulting in a maximal transient increase of the electrolyte resistance. At negative surface charge, electrostatic repulsion occurred; and at high positive surface charge of the artificial wall, it has been suggested that a rearrangement of macromolecules occurs or that positive groups at the erythrocyte surface could be involved in the decrease of the contact efficiency (Godin and Caprani 1996).

Contact time showed a significant dependence on the surface charge density when $q < -6$ μC/cm^2 and $q > +13$ μC/cm^2. This observation was obtained with stored red cells and trypsin-treated red cells, and suggested that dependence of the electrolyte resistance increase on the charge density and the one of contact time could therefore in part originate from the same mechanism.

Significant changes occurred when blood was stored more than 6 days. These results were in agreement with a previous study which indicated that the electrophoretic mobility rapidly decreased during the first week of storage (Leterrier et al. 1979). In the present work, changes were in part illustrated by the progressive decrease in the values of the transient R_e increase at storage duration ≥6 days. Such a decrease was also observed during the experiments with trypsin, which is known to reduce the surface charge of erythrocytes. The reduction in the level of transient R_e increase could thus be related to a surface charge reduction. In addition, some authors have reported that trypsin decreases the surface charge of the erythrocytes by 60% (Böhler and Linderkamp 1993); then, the present trypsin experiments suggested that the surface charge of red cells could be reduced by about 60% at 8 days of storage at 4 °C. After two weeks of storage, the electrical response was independent on the charge density of the microelectrode (no significant difference). This finding suggested that red cells should no longer be charged after two weeks of whole blood storage at 4 °C.

Together with the surface charge reduction, rigidification of red cell membranes should be increased. Loss of deformability can be attributed to an increased internal viscosity and to poorer elastic properties. Normal erythrocytes are discoid with a 2.5 μm maximum and 1.5 μm mini-

mum thickness for the biconcavities (Seaman 1975). They are easily deformable, which facilitates the progression of macromolecular bridge formation when red cells are sufficiently close to an adequate charged support, resulting in an increase of the total area of cell surface in contact with the support (Fig. 7). A 5.0 ± 0.3 µm contact diameter was calculated from Eq. (6) for blood stored up to 3 days. At 6 days of storage, contact diameter remained roughly at the same value (4.4 ± 0.3 µm) which could suggest that the deformability of the red cell membrane should not be significantly affected.

When blood was stored for more than 6 days, rigidification of the red cell membrane could arise, resulting in a less efficient contact. According to a previous study which has demonstrated that only small numbers of crenated spheres and spherocytes can be observed at two weeks of storage (Rumsby et al. 1974) and according to our microscopic observations that erythrocytes remained largely discocyte in shape (data not shown), one can presume that, under our experimental conditions of storage, no important change in the red cell shape occurred which could alter the contact surface area. Then, rigidification was assumed to be the sole phenomenon affecting the cells. In fact, less deformable red cells should create fewer macromolecular bridges, and rigidification of the membrane should increase until the occurrence of current leakage between the cells and the artificial wall at long periods of storage (>10 days). This suggestion, in addition to the surface charge decrease, could explain the relatively low transient increase of R_e obtained at long periods of storage (<1.4%).

The apparent decrease of contact time after 10 days of storage supported this idea. When red cells are rigidified, fewer bridges should be created, resulting in a lower adsorption force. In consequence, the contribution of the shear flow forces should be more important with a tendency to wash away the red cells. Furthermore, owing to current leakage red cells should only be detected when completely on the microelectrode. Then, the effective contact length should be smaller than 20 µm when cells were rigidified. Both phenomena should contribute to artificially decrease the contact time of one red cell with the microelectrode. Trypsin experiments corroborated this point of view; trypsin-treated erythrocytes showed a reduced contact time compared to untreated cells, which shows that trypsin can alter the elasticity of the cells as suggested by Schmid-Schoenbein et al. (1986) or modify the cell deformability as neuraminidase does (Paulitschke et al. 1994).

In consequence, our results underline a possible relation between surface charge decrease and increased rigidification of red cells during blood storage. In fact, the major sialylated glycoprotein of the red cell surface is glycophorin A, which exists in close association with the anion transporter band 3. The modification of these integral proteins can modulate the elastic behaviour of the red cell membrane (Knowles et al. 1994). Furthermore, neuraminidase treatment of erythrocytes, which removes the sialic acid from the erythrocyte membrane, can cause a concentration-dependent rigidification of the cell membrane. High concentrations of neuraminidase could remove a less accessible portion of sialic acid which could alter the interaction between sialylated integral proteins, such as glycophorin A and band 3 (Paulitschke et al. 1994). In our studies, it could be assumed that a two week storage at 4 °C removed the less accessible portion of sialic acid and profoundly altered the cell deformability.

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