Membrane Fusion and Deformation of Red Blood Cells by Electric Fields

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Human red blood cells suspended in a slightly hypotonic solution of low electric conductivity were exposed to an inhomogeneous and alternating electric field (sine wave, 30 V peak-to-peak value, electrode distance 120 μm, 0.5 to 2 MHz). Due to the dielectrophoretic effect the cells align parallel to the field lines under the formation of pearl chains. At high voltages (10 V amplitude) membrane fusion is observed between the adhered red blood cells in the pearl chains, whereby the chains become attached to the electrodes. In contrast to the pearl chains observed at voltages of up to 5 V amplitude the resulting fused and uniform aggregates which exhibit no recognisable individual cells under the light microscope, remain stable, even after the alternating electric field has been switched off or after haemolysis in response to osmotic shock. The fused aggregates are highly elastic. If the field strength of the applied alternating electric field is further increased they are stretched in the direction of the opposite electrode. Frequently, bridges are formed between the two electrodes. The uniform bridges remain stable for some time even in the absence of an electric field. The possibility of cell fusion and its initiation by electrical breakdown of the cell membranes are discussed.

Introduction

Studies of the mechanical deformability of red blood cells are of great interest for both membrane research and clinical applications. Data concerning deformability were derived either from bulk measurements (*e.g.* centrifugation, filtration, viscosimetry, etc.) or from studies of individual cells, using micropipette suction or special flow chamber techniques in combination with optical methods (light microscopy, cinephotomicroscopy, scanning electron microscopy, etc.) [1–8]. Changes in the deformability of the erythrocyte membrane can be caused both by isochoric alterations in cell shape and by changes in the elastic characteristics of the membrane. In addition, investigations carried out on heated red blood cells (about 50 °C) [9, 10] and on red blood cells attached to a glass cover slip in a parallel-plate flow channel and subjected to hydrodynamic shear stress [11, 12] have shown that under certain experimental conditions vesicle and tether formation and fragmentation can occur.

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In this communication we report on deformation associated with membrane fusion of red blood cells in response to a nonuniform and alternating electric field of high intensity. In an alternating uniform electric field both neutral and charged particles exhibit the same behaviour (in contrast to electrophoresis in a uniform field), because the charge of the particle is "masked" by the alternating field and the charged body tends to merely vibrate about its original position. On the other hand, because of the presence of the alternating electric field, a dipole is generated in both neutral and charged particles. Since the forces operating on the two regions in the cell are unequal in a non-uniform field, the result is the creation of a net force. Thus, the cells are steadily pulled into the region of higher field intensity. This effect is termed dielectrophoresis [13]. In addition in red blood cells dielectrophoretic motion results in the formation of "pearl chains", that is an ordered arrangement of the cells parallel to the field lines. This effect is known as mutual dielectrophoresis [13, 14] and it arises from different relative dielectric constants of the cells and their surrounding medium. The field strengths that have so far been applied in dielectrophoretic studies of

red blood cells were not sufficiently high to induce deformation of the cells or fusion. However, as demonstrated in this communication, deformation and membrane fusion do occur when the field strength is high enough to reach the critical voltage required for reversible electrical breakdown [15–19].

Materials and Methods

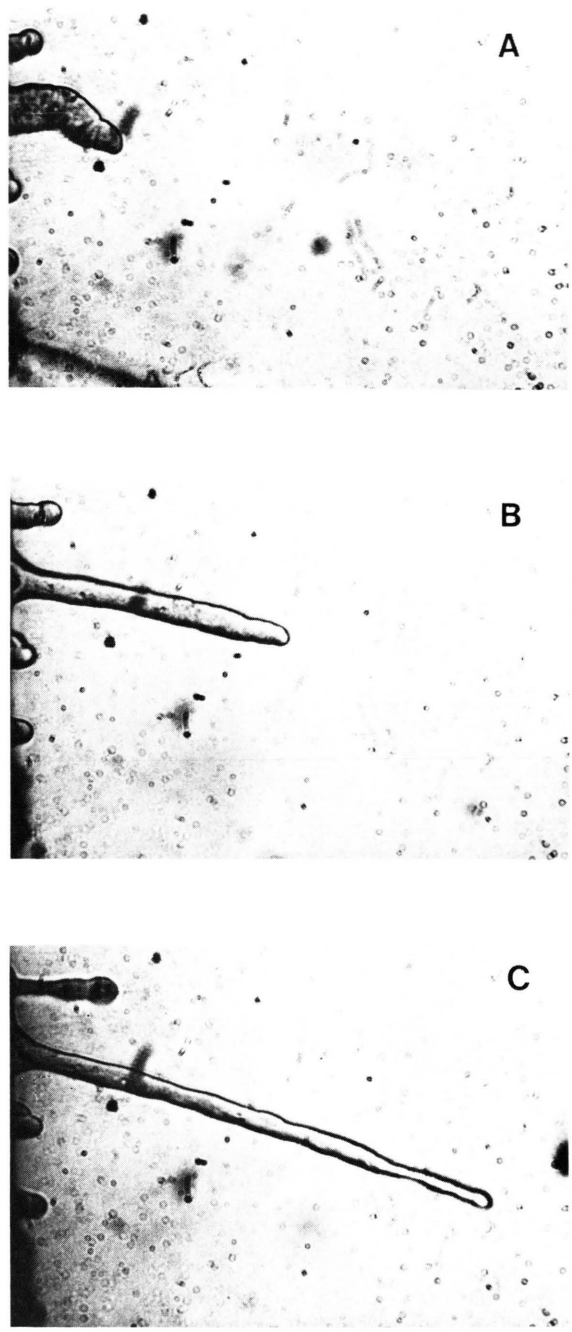
Fresh human red blood cells from apparently healthy donors were used in the experiments. The red blood cells were collected and washed in the usual way [20] and stored in a slightly hypotonic solution containing 230 mM sucrose at 4 °C for a maximum time of 2 h. Electrolyte-free sucrose solution was used, because the dielectrophoretic effect is reduced if the conductivity of the suspension is above 10^{-5} S/cm [13, 21].

For the dielectrophoretic experiments, a slide was used, and two platinum electrodes 0.5 mm thick and 5 mm wide were glued to it in opposition to each other. The two opposite ends were smoothed off, and their radius was 2.5 mm. The minimum distance between the two electrode ends was 120 µm. With this electrode configuration a strong non-uniform electric field can be established in the gap between the electrodes. A function generator manufactored by Toellner, type 7404 P, West Germany, was used for the generation of the alternating voltage.

The voltage applied to the electrodes was varied between 2 and 15 V amplitude and the frequency was varied between 0.5 and 2 MHz. The alternating voltage was monitored on an oscilloscope. For the experiments 200 µl of a 230 mM sucrose solution were injected into the gap between the electrodes, followed by a few µl of a red blood cell suspension (suspension density 1 : 20). The behaviour of the red blood cells in the electric field was followed under a Zeiss photomicroscope fitted with a water immersion objective. the cells exposed to the external fields were photographed with a built-in polaroid camera. The temperature was kept at about 25 °C.

Results and Discussion

After the alternating field is switched on, the red blood cells immediately begin to adhere to the electrodes and to each other in the direction of the



Figs. 1 A to C. Human red blood cells fused in a non-uniform alternating electric field. A voltage of 7.5 to 10 V amplitude (sine wave, 500 kHz) was applied. If a homogeneous electric field is assumed, a field strength of 830 V/cm can be calculated (electrode distance 120 µm). Fig. 1 A shows the field induced fused aggregate after the alternating voltage is switched off. Figs. 1 B and C show stretching of the fused aggregate with increasing amplitude of the alternating voltage (6 V and 12 V, respectively). 1 cm = 32 µm.

field lines. At low voltage (1 to 5 Volt, 80 to 400 V/cm, when assuming a homogeneous field), the well-known "pearl chains" begin to form. Under these conditions reversible cell adhesion occurs. If the electric field is removed, the pearl chains disintegrate immediately because of Brownian motion of the cells. At more intense field strength, on the other hand, the erythrocyte membranes fuse.

Fig. 1 A shows a few red blood cells (viewed under the light microscope) attached to the surface of one of the electrodes, which have fused into a uniform aggregate in the presence of an alternating voltage of 7.5 to 10 V amplitude. Increasing of the voltage between the electrodes leads to increasing deformation of these fused cells, as shown in Figs. 1 A to C. The deformability of these aggregates consisting of only a few cells is quite considerable. As Fig. 1 C demonstrates, the cell aggregates can be stretched by more than a factor of 3 at a voltage of 12.5 V amplitude. This field-induced stretching is reversible, and a decrease in the electrode voltage leads to an contraction within seconds of the stretched cell aggregates. Subsequent increase of the electrode voltage even when repeated more than 20 times, does not appear to cause any changes in the elasticity of the cell aggregates. Removal of the electric field shows that the contact between the cell membranes is tight. The cells cannot be separated from one another, not even when they are subjected to osmotic shock.

In some cases almost spherical aggregates are obtained (consisting of up to 50 erythrocytes), which are also stable in the absence of the non-uniform and alternating electric field (Fig. 2) and do not disintegrate into individual cells even though when they are subjected to osmotic shock. In the case of tight adhesion of several cells it is possible to stretch the chains to the extent where they will attach themselves to the other electrode surface, by applying high electrode voltages. Under the light microscope these bridges look completely uniform (Fig. 3) and individual cells are no longer recognisable. These cell bridges are shown to be stable even in the absence of the electric field. If the same experiment is carried out at low voltages or in the presence of small amounts of an electrolyte ($CaCl_2$), pearl chains may be formed between the two electrodes, but the individual cells within these chains are still identifiable as independent units under the light microscope. If the electrode voltage is switched off, these bridges disintegrate completely into the individual component cells.

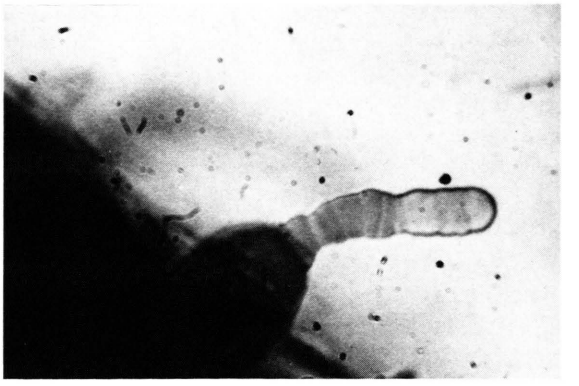


Fig. 2. Fused red blood cell aggregate formed of about 50 erythrocytes in an alternating electric field. The picture shows the aggregate after the fusion process was completed and the alternating voltage was switched off. 1 cm = 19 μm.

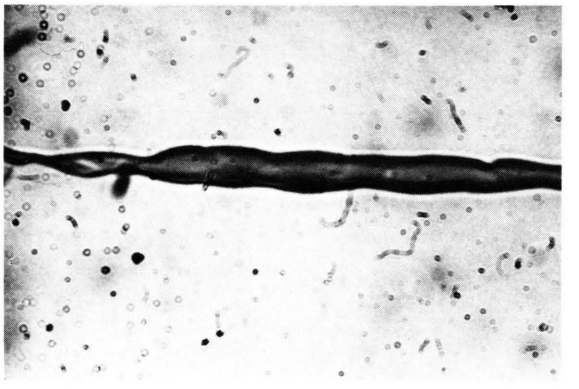


Fig. 3. "Bridge" of fused red blood cells between the two electrodes. The bridge was obtained by stretching a smaller fused aggregate in the presence of an alternating electric voltage of 15 V amplitude. The bridge remained stable without applied electric field for some time. Note, that no internal cell compartements are visible. 1 cm = 19 μm.

If the osmolarity of the external medium is progressively lowered after the formation of stable bridges consisting of irreversibly attached cells, individual compartments in these chains are found to haemolyse. The outcome of this experiment could indicate that complete cell fusion did not occur in this case. On the other hand, it is quite possible, that the chain broke at a certain place because of the high elastic tension and that it immediately sealed itself off again. This assumption is supported

by the experimental finding that in bridges consisting of only a few cells, *i. e.* bridges under high elastic tension, the bridge often ruptures spontaneously at one point and both ends seal themselves off immediately, without any visible loss of haemoglobin.

It is thus impossible to ascertain at present whether cell fusion occurs as a result of the electric field. Zimmermann and Pilwat [22], (see also [23]), reported that cell fusion can occur at low yield when subjecting a red blood cell suspension in a discharge chamber to electric field strengths sufficiently high to induce electrical breakdown of the cell membrane [24]. The reasons for the low yield of fused cells under these conditions are quite obvious, since cell membrane contact seems to be the most important prerequisite for cell fusion [25–27]. Brownian motion, however, and repulsion of the charged cells reduce cell membrane contact.

The process of membrane fusion described in this communication should be primarily attributable to an electrical brakdown of the cell membrane [17]. If one assumes that the field is roughly homogeneous, in a first approximation then the field strength at which the irreversible formation of uniform aggregates is observed under the light microscope, is estimated to be about 625 V/cm. On the basis of the integrated Laplace equation [28] this would correspond to an induced membrane potential difference of 0.3 V. The breakdown voltage for red blood cells is of the order of 1 V. However, at the surfaces of the electrodes the external field strength should be considerably higher, that is by a factor of 10 or more [13]. In addition, the field strength close to the membrane surface should be much higher due to mutual dielectrophoresis. At these field intensities, large areas of membrane would be subject to breakdown [28]. This seems to be an important prerequisite for the observed membrane fusion. The fact that the membrane fusion begins between cells attached to the electrodes and then continues into the gap between the electrodes by fusion with other cells agrees well with this concept. The field intensity required for the breakdown within the growing fused cylindrical aggregates along the axis of the field lines decreases in proportion to the long axis of the cylindrical fusion aggregate [13].

The effects of fields on the deformability of red blood cells are somewhat comparable to those described by Harbich and Helfrich [29] for lipid vesicles, which deformed into elongated cylinders under the influence of electrical fields. If the course of the electric field within the gap between the electrodes is known, it may be possible, in future, to make quantitative estimates of the elastic behaviour of the erythrocyte membrane in the electric field. Further studies using more sophisticated techniques should also reveal whether cell fusion actually occurred in the experimental set-up described here. If fusion did occur, then this physical technique of cell fusion should, in principle, be applicable to other cells, since all living cells exhibit the phenomenon of dielectrophoresis and electrical breakdown of the cell membrane [13, 17].

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