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MAGNETO-ELECTRO-FUSION OF HUMAN ERYTHROCYTES

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In inhomogeneous (static) magnetic fields close contact between 'magnetic' human erythrocytes was established. The cells were made magnetic by incubating them in a medium containing small Fe_3O_4-particles which adsorbed to the outer membrane surface. Fusion was induced by applying two electric field pulses (field strength: 8.5 $kV \cdot cm^{-1}$; duration: 60 μs) to the magnetically collected cells. This procedure allowed the use of electrically conductive media ($3 \cdot 10^{-3}$ $\Omega^{-1} \cdot cm^{-1}$). Fusion of red blood cells occurred very often. If cell suspensions of high density were used fusion resulted in the formation of giant red blood cells with osmotically intact membranes.

Reversible electrical breakdown of cell membranes leads to fusion of cells provided that an intimate contact between the cells under consideration exists [1–5]. Membrane contact between suspended cells can be achieved either by suspending cells at high densities [6,7], by using chemicals [8] (or viruses), or by aligning cells in inhomogeneous electric fields (so-called dielectrophoresis, [1–5]). The vectorial character of electric fields has the consequence that the dielectrophoretic effect was previously the only practical method of determining the number of cells to be fused, or indeed of controlling the entire fusion sequence before and after the breakdown pulse. The so-called 'electrofusion' method therefore permits the fusion of plant, animal and microbial cells, liposomes and eggs under controlled conditions. Suitable equipment is now commercially available and there exists a series of reports about electrofusion leading to viable hybrids which undergo growth and division [9–12].

The basic requirement for fusion between two cells is the establishment of close membrane contact. This may be achieved besides by electrical forces also by other physical forces. The use of mechanical forces combined with an electric breakdown pulse has been reported [13]. However, such a technique was (at least to the authors' present knowledge) only applicable to larger cells such as plant protoplasts and requires manual skill. Additionally, the number of cells to be fused is constrained to two. In this communication we report on a magneto-electro-fusion technique which uses magnetic forces to bring about close contact between cells which have previously been made magnetic. The application of inhomogeneous magnetic fields offers both the abilities to precisely control the process of fusion and to perform cell fusion in electric conducting solutions. Within the hitherto existing technique of electrofusion non-conducting or weakly conducting solutions had to be used. Though it was shown there were no adverse side effects on the viability of the cells if they were incubated in these solutions for not longer than 30 to 60 min, there are advantages in performing experiments in electrically conductive media because such work may provide an answer to the question of the mecha-

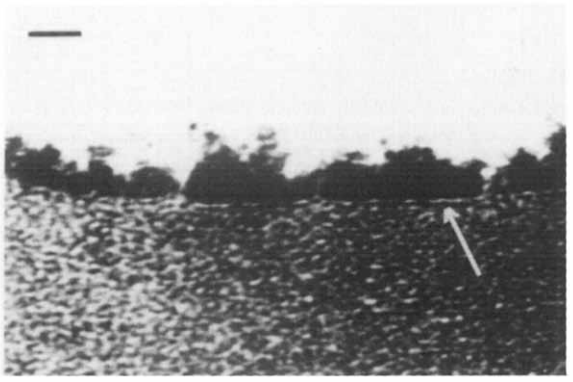

Fig. 1. Part of a human red blood cell with Fe_3O_4-particles adsorbed to the surface of the cell membrane (arrow) forming a black electron-dense coat. Grey parts represent hemoglobin. Electron-micrograph, bar = 0.1 μm.

nisms involved in electrofusion [2,3].

The experiments were done on human erythrocytes which were prepared as follows. Blood was taken from apparently healthy donors, centrifuged at 1500 × g for 5 min and the serum and buffy white coat were removed. The erythrocytes were washed twice in an isotonic NaCl solution and then incubated in an isotonic solution containing 280 mM (glucose or) saccharose, 10 mM KCl, 1 mM $MgCl_2$ (Sol I) and magnetic particles. Commercially available 'Ferrofluid' solutions (Ferrofluid Corp., Nashus, NH, U.S.A.) were used for making the cells magnetic. These solutions consist of Fe_3O_4 particles as a suspension stabilized by anionic surfactants. As described elsewhere [14], before adding the 'Ferrofluid' solution to cell suspensions these surface active agents have to be removed; otherwise they lead to haemolysis. For this reason the Ferrofluid solutions were shaken (2.5 h) with Amberlite MB 2 ion-exchange beads (Serva GmbH, Heidelberg, F.R.G.) until their conductivity was reduced to $10^{-5}\ \Omega^{-1} \cdot cm^{-1}$. Then the Fe_3O_4 solution was filtered through a Millipore filter (0.45 μm pore width). Five percent of this suspension was added to the Sol I solution which was supplemented with various amounts of isotonic NaCl solution. The red blood cells were incubated in one of these solutions for 20 min at 37 °C. Under these conditions magnetic particles were adsorbed to the outer membrane surface, which are seen on electron-micrographs as a black electron-dense layer (Fig. 1). For these micrographs the 'magnetic' cells were fixed in 2% glutardialdehyde, dehydrated in a graded alcohol series, embedded in Spurr's epoxy resin, and viewed on a Jeol electron-microscope JEM 100 C. For removing excess Fe_3O_4 particles and also those particles not tightly adsorbed to the outer membrane surface the suspension of erythrocytes and Fe_3O_4 particles was diluted with isotonic NaCl solution (1 : 1) and centrifuged (1500 × g, 3–4 min). The pellet was then washed in isotonic NaCl solution, centrifuged and again incubated in the above-described solutions (their conductivity was up to $3 \cdot 10^{-3}\ \Omega^{-1} \cdot cm^{-1}$). Immediately before the 'magnetically marked' erythrocytes were brought into close contact by application of an external inhomogeneous magnetic field pronase or neuraminidase (Serva GmbH, Heidelberg, F.R.G. and Behring Werke AG, Marburg, F.R.G.) was added at a concentration of 1 mg/ml or 0.2 U/ml, respectively, to remove the glycocalix on the cell surface. The fusion was carried out at room temperature without removing the enzyme.

For alignment of the 'magnetic' cells the following set-up was used: Four iron-silicon sheets (as used in transformers) were glued together in the shape of a cross as shown in Fig. 2. The distance between the poles was adjusted to 12.5 μm by means of Kapton films (E.J. du Pont de Nemours & Co., Wilmington, DE, U.S.A.) of suitable thickness. The poles were covered with a foil consisting of aluminium, one side of which was insulated. On top of this film two platinum wires (diameter: 150 μm) which serve as electrodes were arranged in parallel, either about 200 μm or 500 μm apart (as shown diagrammatically in Fig. 2). A magnetic-field gradient is induced in the area of the cross by applying 5 V to two independent coils with iron cores (number of windings: 1700; $R = 32\ \Omega$) which 'sit' at points denoted by 1,1 and 2,2 in Fig. 2. Any magnetic particle (e.g. a 'magnetic' blood cell) experiences forces in these magnetic gradients so that it moves towards the 'gaps'. The geometry not only causes an alignment of magnetic particles but also gives rise to close contact between the particles because there is a moderate force directed to the mid-point.

The fusion of the magnetically collected cells was triggered by two electric field pulses with an

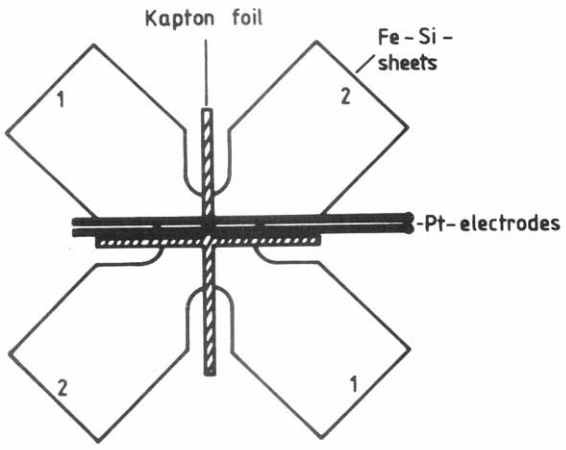

Fig. 2. Scheme of the chamber used for magneto-electro-fusion. Thin Fe-Si sheets are glued on top of a support. The constant distance of 12.5 μm between the four sheets is achieved by means of Kapton films. On top of the sheets two Pt electrodes (diameter 150 μm) are arranged about 200 μm apart from each other and separated from the metal sheets by means of an insulating foil (not shown). Points denoted by 1,1 or 2,2 are connections for the two magnetic circuits each containing a coil.

interval of some seconds. The pulse length was 60 μs at a voltage of 170 V (corresponding to a field strength of 8.5 $kV \cdot cm^{-1}$).

Under the experimental conditions described fusion of human red blood cells was observed. Fusion products formed from two or three cells were frequent but giant cells also appeared regularly if high suspension densities were used (Fig. 3). All fused cells proved to be stable in 'Basal Medium Eagle' (Boehringer Mannheim GmbH, F.R.G.) supplemented with 10% calf serum. This indicates that the membranes were osmotically intact and indeed no significant haemolysis was observed.

These erythrocytes having magnetic particles adsorbed to the membrane surface required higher field strengths to trigger the fusion process than the untreated blood cells in mannitol solution after dielectrophoretic collection [15]. As could be shown by measurements in a Coulter Counter the 'magnetic' cells apparently possess a membrane breakdown voltage about 40% higher than that of normal erythrocytes [1,16]. The reasons for this are

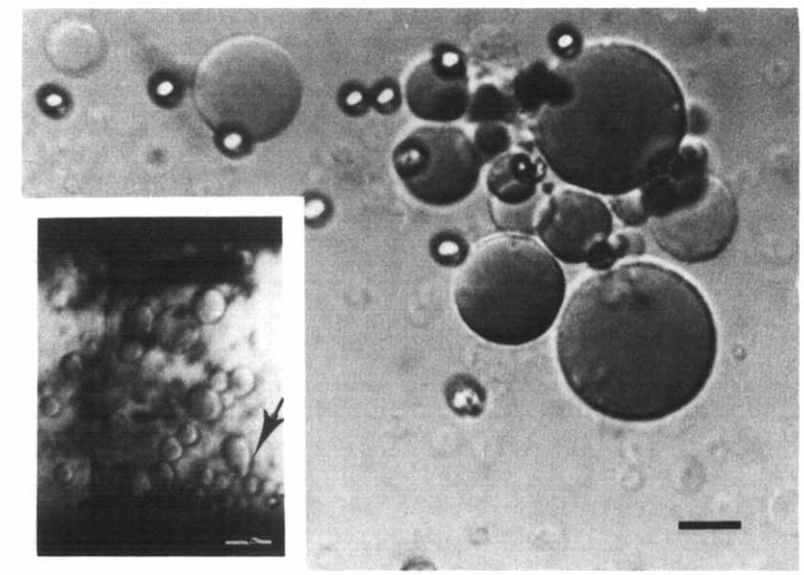

Fig. 3. Giant red blood cells obtained by magneto-electro-fusion of thousands of cells. After the fusion process is complete, cells are withdrawn from the chamber and suspended in isotonic 'Basal Medium Eagle' medium on a separate microslide. The giant cells are surrounded by erythrocytes which were not exposed to the electric field pulse. Bar: 100 μm. Inset. Human red blood cells coated with magnetic Fe_3O_4 particles are collected between two Pt electrodes (black lines) within one minute of magnetic field application. Injection of an electric field pulse of 8.5 $kV \cdot cm^{-1}$ and 60 μs duration leads to fusion. The micrograph which was taken about 30 s after the electric field application shows fusing human red blood cells which still increase in size. This is evident from the shape of some giant cells which have not yet become spherical (arrow). Bar: 100 μm.

not yet clear. As can be seen from the electron-micrographs the Fe_3O_4 particles form almost a closed shell around each cell. It seems likely that this semi-conducting 'coat' has an influence on the electric field distribution around the cells. It also has to be considered that under the conditions described here for 'magneto-electro-fusion' the breakdown in the membrane may not be of purely electric character. The adsorbed Fe_3O_4 particles may partly penetrate the membrane if the blood cells come into close contact. Hence pores may be formed mechanically and fusion (for which lined-up pores in apposed membranes are prerequisite) would be partly initiated by mechanical means. This possibility warrants further attention in future.

To sum up, the described technique seems to be an alternative way to bring about controlled cell fusion. The immediate advantage is that conducting media may be used. If during the fusion process magnetic particles are brought inside the forming cells this could simplify the separation of fused and unfused cells as was described recently for liposomes [17]. There is experimental evidence in the literature that small magnetic particles do not have adverse side effects on biological systems (quoted in Ref. 14). So the magneto-electro-fusion technique may be of advantage for large-scale hybrid production.

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