Properties of giant cells formed by electro-fusion

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Abstract—**Pulsed electric fields applied to biological cells have been shown to cause membrane destabilisation or breakdown, and also cell fusion if the cells had been brought into membrane contact (e.g. by dielectrophoresis). In the case of a dense suspension of cells, tens, hundreds or even thousands of cells may be fused into a single giant, multinucleate cell of approximately the same total volume. This giant is usually seen to round up, although spherical geometry demands that it must contain excess membrane area. Experimental data on the membrane capacitance per unit area are in accordance with much of the excess membrane forming folded structures. Some of these may be visible by optical microscopy. That is, these cells have apparent membrane capacity values (capacitance per unit of *visible* membrane area) which are several times that of the original smaller cells.**

I. Introduction

Electrofusion is a technique that has found application mainly for the formation of hybrid cells formed from perhaps 2 or 3 parent cells. An example of these are the hybridoma that can be formed from splenic B cells when fused with myeloma cells: the aim being to combine the properties of antibody production and unlimited growth. In such cases many fusion products survive at least a few generations and may form permanent cell lines, and indeed the electrical technique continues to show some advantages over chemically-induced fusion [1-3]. On the other hand, fusion of large numbers of cells to give a single product is an experimental feat that was initially chiefly a demonstration of capability and of photographic value. Indeed the first such demonstration used circulatory mammalian erythrocytes, which have no nuclei [4,5] and hence could not grow further. Subsequently giant cells were electrofused from an erythroblast cell line ("Friend" cells), which are nucleated [6]: however no measurements of the membrane properties of either of these cell types appear to have been made.

In this paper, passive electrical membrane properties of giant cells at normal osmotic strength (300 mOsm), will be presented: some of these were published before [7]. Passive electrical membrane properties of giant cells in hypo-osmotic media are presented in a parallel paper [8].

II. ELECTROFUSION

The giant cells examined here were produced by electrofusion of SP2 mouse myeloma cells in hypo-osmolar (100 mOsm) media, followed by transfer to normal isotonic (300 mOsm) medium if required. For initial work, including microscopic observation of the dielectrophoresis and fusion processes, the fusion chamber consisted of two parallel platinum wires on a glass slide. Where larger numbers of products were required, the helical chamber [9, 10], see Fig 1, was used. A fusion power supply from the former GCA Precision (Chicago) was used to provide dielectrophoretic voltage to form chains and larger aggregates of cells before applying a fusion pulse largely as described before [6].

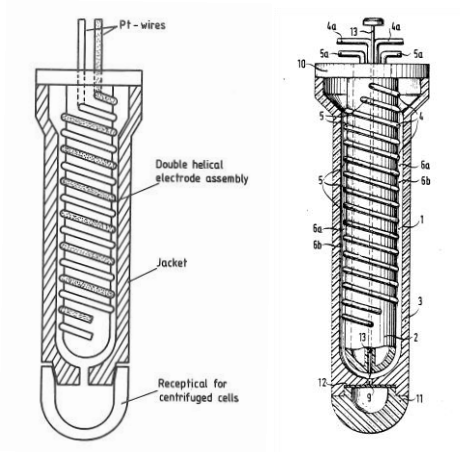

Fig. 1. Helical chamber for the electrofusion of large numbers of cells. In the simpler version (left) a double-helix of platinum wires (200 micron diameter, about 1 m total length) is wound around a PMMA (Plexiglas) cylinder [9]. It is introduced into an outer jacket, in which it is a close fit, and which contains the cell suspension that then fills the inter-electrode spaces. After dielectrophoretic collection, and pulsing to give fusion, the products can be centrifuged into the receptical (*11*) which may initially be isolated via a pierceable membrane (*12*) as shown on the right [10]. Also as shown on the right, the electrodes may be arranged as loops (*4-4*, *5-5*) to provide better voltage distribution (i.e. to mitigate the voltage drop-off that occurs along a lossy transmission line). The whole can be ethanol-sterilized before use with, and dried in a sterile flow-cabinet. The disposable membrane and cup were designed for additional sterility.

III. EXPERIMENTAL

SP2 mouse myeloma cells from a culture 2 days after passage were freed from growth medium by centrifugation at 200 g. Two washes using a hypo-osmolar fusion medium (100 mOsm inositol solution containing 1 µM calcium acetate) and centrifugation were used, after which enough fusion medium was added to give 7.10^6 cells per ml. 360 µl of this suspension were loaded into the helical chamber, and 250 V/cm at 2 MHz applied for 30 s of dielectrophoretic collection, followed by a square unipolar pulse of 1750 V/cm and 15 µs duration. The 2 MHz field was immediately re-applied for 30 s. 10 minutes after field switch-off, the fusion products were used for rotation experiments [8] or transferred to growth medium (RPMI 1640, no dye). They were useable for several days, if

kept in a CO_2 incubator at 37ºC. Such a cell preparation is shown in Fig. 2, a high-reolution view in Fig. 3, and a size distribution is given in Fig. 4.

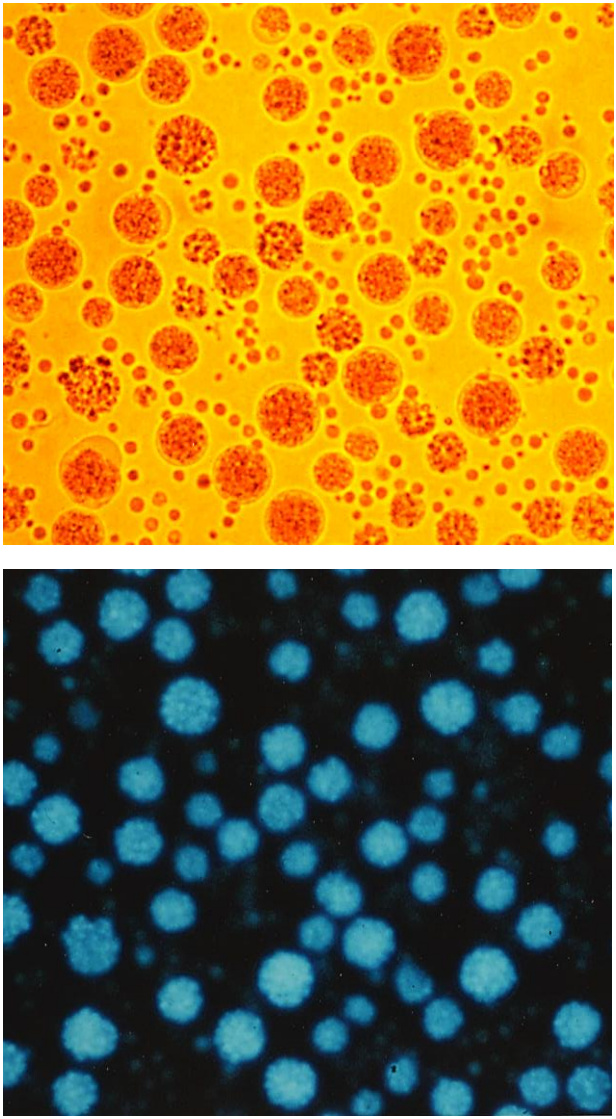

Fig. 2. A preparation of giant, electro-fused SP2 cells: (*above*) transmitted-light and (*below*) fluorescence micrographs of the same view-field and magnification. Each fluorescent dot in the lower picture is one of the many cell nuclei (up to thousands) enclosed in each giant cell. The larger cells are 45-50 μm in diameter: some unfused cells are visible in the upper panel. Almost all cells are closely spherical.

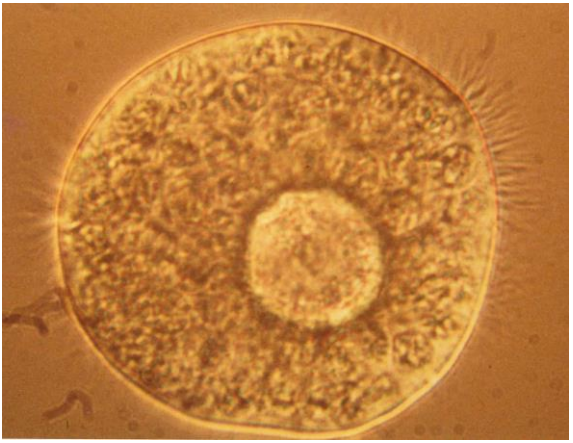

Fig. 3. A giant cell, with a diameter of 62 µm, as it appeared in 300 mOsmol growth medium about 60 minutes after fusion. Some areas exhibit long membrane processes, and apart from the large central structure of unknown formation, a number of smaller, roughly-circular internal structures (nuclei of the constitutent cells) are also visible. Cells up to almost 300 µm were sometimes observed in the parallel-wire chamber.

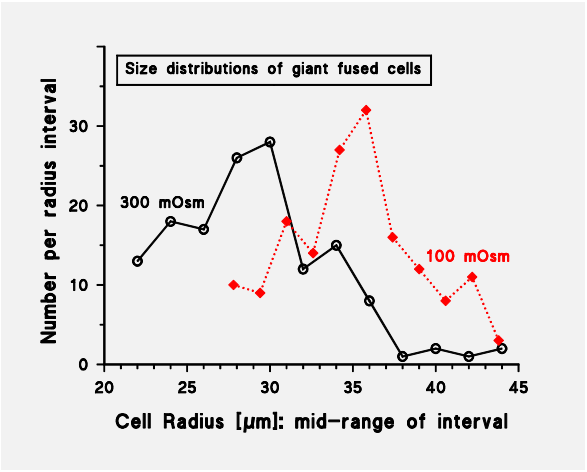

Fig. 4. Size distributions of the giant electrofused cells used for rotational analysis of membrane properties in a parallel paper [8]. 143 cells were measured in 300 mOsm medium, another 160 cells in 100 mOsm medium.

IV. Rotation

Rotational spectra (Fig. 5) were taken to confirm the existence of a symmetrical anti-field rotation peak following the form (Lorentz equation):

$$\Omega = 2\,\Omega_0\, f_n / (1 + f_n^2), \quad \text{with} \quad f_n = f / f_c \tag{1}$$

where Ω_0 and Ω are the peak rotation speed and rotation speed at the normalized frequency f_n respectively. The frequency giving peak rotation speed is f_c, and symmetry about this peak is required for the counter-field rotation technique [8,11] to work as expected.

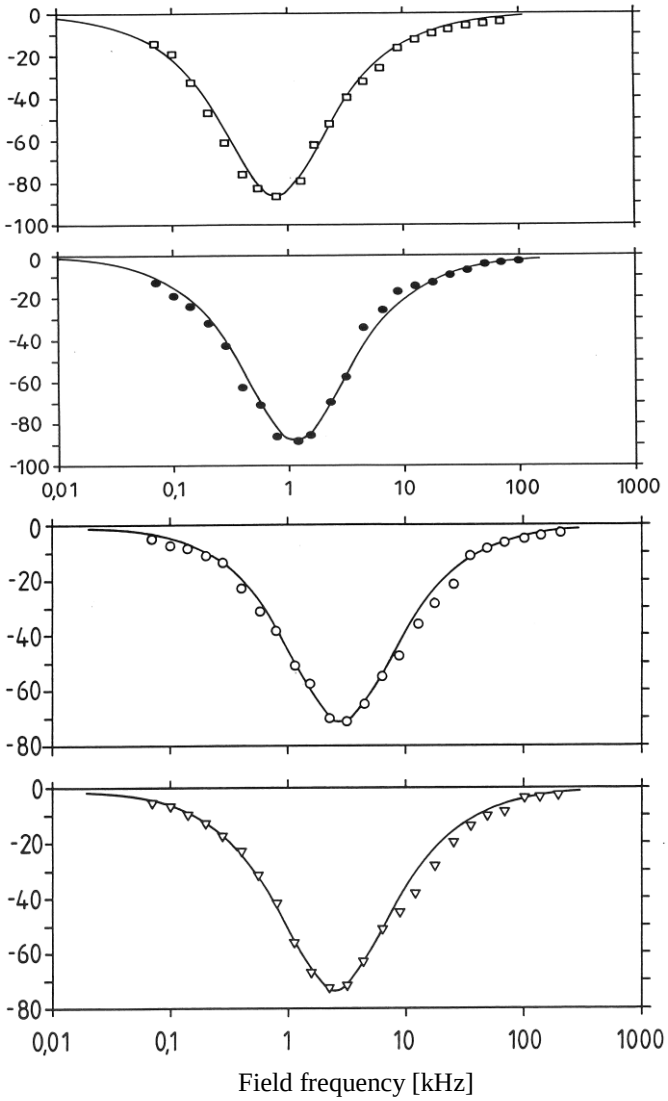

Fig. 5. Rotation speeds, in degrees per second, of four giant cells in 100 mOsm inositol with small amounts of KCl added. The lines drawn through the data are plots of Eq. 1 based on values for Ω_0 and f_c obtained by inspection. The cell radii and medium conductivities were, in order from the top: 49.8 µm in 12.0 µS/cm; 35.3 µm in 12.4 µS/cm; 40.9 µm in 42.6 µS/cm; 42.6 µm in 43.3 µS/cm. The peak frequency increases with conductivity as expected [11].

A field strength of 35 V/cm was used because higher strengths led to membrane disruption at the lower frequencies. Even with this low field strength, for a 50 µm diameter cell, the induced membrane potential, at frequencies well below the f_c, is 130 mV peak.

The spectra show very good agreement with Eq. 1, so that the characteristics of these cells could be measured using the counter-rotating field method [8,11].

V. SINGLE CELL MEMBRANE DATA

For cells which are large enough to be easily seen under a dissection microscope, it is possible to measure the rotation of just one cell at several different conductivities [12]. Washing of the cell can be accomplished by transferring it by micro-pipette between droplets of medium on a Petri dish - without losing sight of it.

The frequency of the rotation peak is measured at each conductivity, and by use of the equations presented before [11] and a linear regression, values for the membrane capacity and conductivity may be derived. The cell radius is also required.

Use of this technique on giant cells resulted in the data of Table 1, and later work [7] extended this to 51 cells: data on these is reproduced in Fig. 6.

TABLE 1: RESULTS OF SINGLE CELL, MULTIPLE CONDUCTIVITY, ROTATION

Cell radius [µm]	Number of conductivities and f_c values	Correlation coefficient of regression	Membrane capacity, C_M [µF/cm^2]	Membrane conductivity, G_M [mS/cm^2]
29.4	4	0.9959	3.29	5.5
28.2	3	0.9999	3.31	4.5
29.4	3	0.9992	2.45	2.0
38.4	4	0.9979	2.71	5.7
37.8	3	0.9998	2.98	3.2
26.9	3	0.9996	2.81	3.1

The extremely good correlation coefficients in Table 1 give grounds to have confidence in the derived membrane parameters. However, the values vary widely, extending from just above that of the starting material to C_M values of 5.5 µF/cm^2. As visible in Fig. 3, these giant cells possess surface villi which may not be evenly distributed. It is believed that these magnify the true membrane area above that expected for a smooth sphere of the same radius. It can also be noted that the largest possible C_M values seem to increase with the radius of the fusion product, at least up to 27-28 µm.

Now the fusion of 4^3 cells of radius 7 µm, at constant volume, should give rise to a cell, if spherical, of radius 28 µm. Indeed the giant cells are observed to "round up" well (Fig. 2). Due to the increase in the ratio volume: area as the size of a sphere increases, the membrane excess is 4:1. In agreement with this, the maximum C_M values seen are 4-5 times those of unfused cells. In such cells, all the available membrane contributes to electrically-accessible villi or other structures. In other cells, some of the membrane is internalized, very tightly stacked, or lost [7].

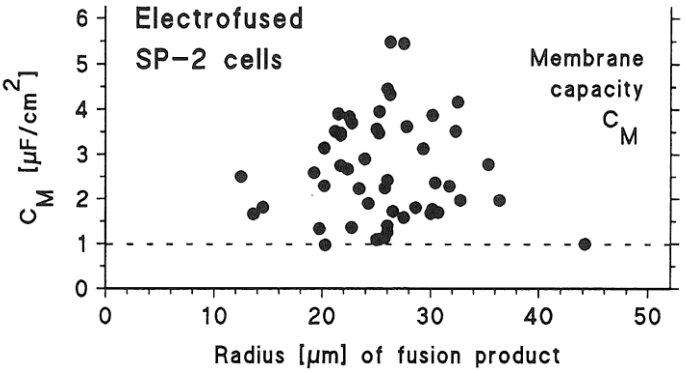

Fig. 6. Membrane capacity values and radii for 51 giant cells obtained by electrofusion in later work [7] © the Biochemical Society. The dashed line shows the C_M value of SP2 cells before fusion.

VI. Acknowledgements

Grateful thanks are due to Prof. U. Zimmermann and colleagues at the Dept. of Biotechnology at the University of Würzburg where these measurements were carried out. WMA thanks the MacDiarmid Institute of Victoria University and Callaghan Innovation for travel finance and permission to attend the ESA Conference.

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