Improved membrane measurements from diverse cell populations

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Abstract—**Electro-rotation is a visually-based technique that can be applied to a wide range of biological cells, as will be shown. Compared to techniques such as dielectrophoresis or impedance spectroscopy, it is far easier to apply to single cells. It has been extensively used to extract the passive electrical membrane properties, such as the membrane capacitance per apparent unit area, from electrically-driven cell movements. These properties, which are strongly affected by membrane microstructure, are very useful as indicators of cell type, proliferation in both normal and cancerous states, and cellular health within a cultured or *in vivo* population. Highly reproducible measurements can be obtained on individual cells, yet in some cases the correlations that can be made between data from sets of cells are relatively poor. A new approach to examining this diverse-cell data is presented here, and some examples of its use are examined. In terms of biological data, it is only now possible to see uniformity of membrane properties despite the extreme size differences of some cellular populations.**

I. INTRODUCTION

Rotation of cells in electrical fields was seen during early work on the dielectrophoretic collection of cells, so that it was considered to be induced by a linear field until Holzapfel et al. [1] realized that the phase shifted dipole moments in adjacent but suitably-positioned cells were each generating a rotating field component. *External* application of a rotating field enabled rotation of single cells to be observed, so that cell-cell interactions were excluded and relatively precise measurements could be made [2-4]. This was particularly the case for round, smooth cells such as protoplasts made from plant cells, and also certain membrane-bound vesicles [5].

Examination of the frequency spectrum of single cells showed the rotation direction to be opposite to the field direction ("anti-field rotation"), at least over the majority of the accessible frequency range. This was explained in terms of the changing polarizability of the cells in that frequency range. This polarizability is large at low frequencies, and its origin is in the charging of the cell membrane for which ions from the medium are required. Hence in low-conductivity media this process becomes phase-shifted with respect to the applied field and a torque is developed as the field rotation rate is moved up through the kHz range.

Observation of the rotational spectrum in response to variation of the medium conductivity can then be used to separate the various membrane properties that contribute to the charging time. Of these the area-specific membrane capacitance is often quoted, and is usually found to exceed that of model (flat) membranes by a factor that can be considera-

ble. In many cases, a large excess factor could be correlated with the presence of known membrane foldings, villi etc [6-8]. These are of biological significance: increases in membrane capacity were observed when growth-stimulation signals were applied to cells such as oocytes immediately after fertilization [9], and also in T and B lymphocytes following mitogenic stimulation [10]. Cells from tumor cell lines, being in a state of permanent activation, might also be expected to exhibit unusually high values of membrane capacity: this has indeed been found to be the case by both dielectrophoresis [11] and electrorotation [12].

II. ELECTROTATION METHOD

Theoretical prediction of the rotation spectra of particles can be made by considering the interaction of a rotating field with the frequency-dependent dipole that it induces [13]. Spherical shelled particles, such as many biological cells when rounded up in suspension, can be modeled as equivalent homogeneous particles. A set of such predictions are shown in Fig 1, and these are closely borne out by experimental spectra.

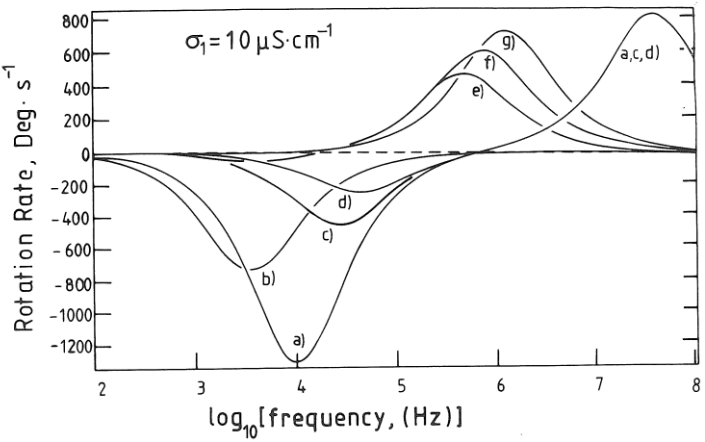

Fig. 1. Rotation spectra predicted for spherical cells and shells in a medium of low conductivity. Curves a) to d) are predicted for biological cells (modelled as a bilayer lipid membrane enclosing a relatively conductive interior). Only the "anti-field" rotation peak in the 3-30 kHz region is normally measurable and provides the data considered below. Curves e) to f) are predicted for relatively conductive shells which are not considered here. For further details, see Ref. [14]: note that σ_1 in that work is termed σ here.

Membrane biologists prefer to use the concepts of specific membrane capacitance (C_m) and conductance (G_m) instead of the strictly dielectric parameters permittivity, conductivity and thickness. In addition, at least for cells of radius less than a few microns, the external surface conductance (K_s) - the conductance tangential to the surface - may cause a similar or greater effect than the membrane conductance.

The properties of the cell membrane can be determined by curve-fitting to the anti-field peak in a measured rotation spectrum. The cell radius (a) will also be required, and to remove uncertainties in the multi-parameter fitting process, it is advantageous to combine

the data from more than one medium conductivity (σ). Alternatively, and much more readily, the peak frequency (f_c) and cell radius alone can be measured at a number of conductivities, in which case the equation:

$$f_c.a = K_s/\pi a C_m + aG_m/2\pi C_m + \sigma/\pi C_m \quad (1)$$

is applicable [15], providing that the medium conductivity is much less than that of the cell interior. As the latter is 4 mS/cm or typically even higher, medium conductivities up to about 80 µS/cm are useable. For many cells, such low-electrolyte media have undesirably low osmotic pressure, so that sufficient concentrations of non-ionic solutes (inositol was used here) must be added to the medium .

Equation 1 predicts a linear dependence of peak frequency multiplied by cell radius on medium conductivity, and this relationship has been used extensively to derive cell membrane properties [6-10, 12, 15-17]. A linear regression of $f_c.a$ on σ delivers a value of C_m from the gradient. The intercept on the $f_c.a$ axis gives the sum of the two conductive terms (involving K_s and G_m), although subject to larger relative errors than the gradient.

Early difficulties in measuring the exact peak frequency were overcome by use of two contra-rotating fields of equal amplitude [15,18,19]. This converts the broad peak (Fig. 1 has a logarithmic frequency axis) into its differential, so that f_c is detected as a null point (Fig. 2) which can often be measured with a resolution of 1%.

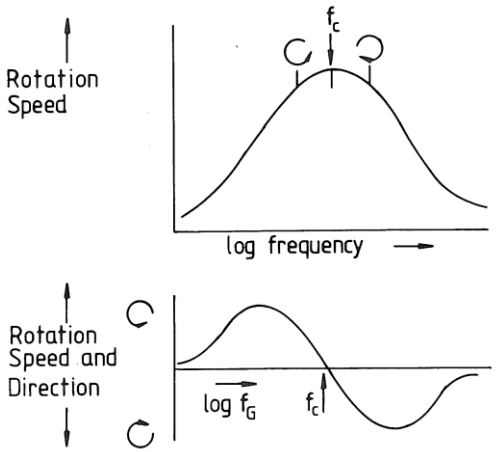

Fig. 2. The contra-rotating field method uses two equal but opposite rotating fields. When scanned across a rotation maximum (upper diagram), the resultant rotation spectrum (lower diagram) is the derivative of the broad rotation maximum (which is itself the derivative of a dielectric dispersion [13]). The ratio between the two field frequencies has usually been 1:4 for technical reasons. However, optimum sensitivity at the zero-crossing point is expected [19] at a ratio of 1:5.83, although the enhancement above that from 1:4 is only a few percent.

Despite this fine resolution, the scatter of rotation data taken even from apparently very similar but size-diverse sets of cells is often 10 or 20% of the mean value. Whilst some of this may be biological variability, some may be due to the way the data is being presented or processed.

A possible limitation with the use of Equation 1 occurs when cells of a wide range of sizes are measured and analysed together. Ideally, if the membrane properties of the cells are invariant with size, analysis should not depend on the size profile of the sample. The terms in K_s and G_m are both radius-dependent, so that the contribution of each individual cell to the $f_c.a$ intercept will be weighted according to its size. This is not consistent with the concept of a well-defined value for the intercept, and can also be expected to worsen the statistical correlation of the data to a straight line "best-fit".

A better approach, at least in some cases, might be the use of the equivalent equation

$$f_c = K_s/\pi a^2 C_m + G_m/2\pi C_m + \sigma /\pi a C_m \qquad (2)$$

as the basis of a linear regression, i.e. of f_c on σ/a. In this case the contribution to G_m, deduced from the f_c intercept, by cells of various sizes but similar G_m should be constant. On the other hand, the contribution to the f_c intercept from the K_s will be strongly radius-dependent, but this contribution is expected to be small for cells of radius greater than about 5 microns. The expectation that Eq. 2 should out-perform Eq. 1 has been tested on data from three types of cell. To enable a fair comparison, linear regression analyses were performed on the complete set of cell data, without preliminary derivation of means at each conductivity (as is sometimes done).

III. RESULTS

a) Yeast protoplasts

Yeast cells grow a cell wall and are usually not spherical: both characteristics make quantitative interpretation of their membrane properties difficult. Linear relationships such as Eq. 1 or Eq. 2 appear, because of the cell wall chemistry, not to hold [20]. However the cell wall may be removed enzymically to give protoplasts [21]: these have the advantage of being closely spherical, especially if swollen in a hypotonic medium.

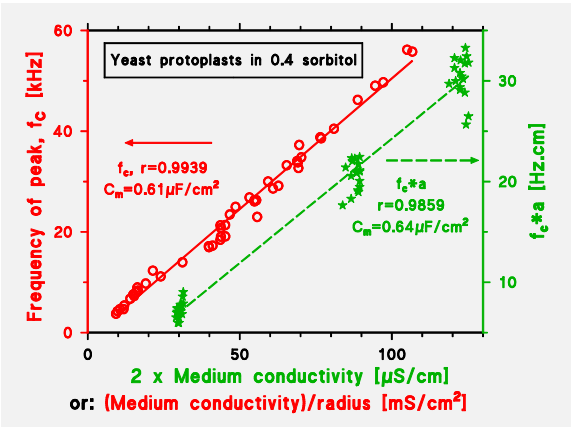

Fig. 3. Rotational measurements of 50 yeast protoplasts, plotted using linear regressions according to Eq. 1 and Eq. 2, with comparison through correlation coefficients *r*. The clustering of points in the $f_c.a$ plot reflects the experimental technique of measuring at three nominal conductivities. In the f_c plot, normalization against the cell radius spreads the identical set of data points out along the regression line. Intercepts were both negative: -1.38 Hz.cm (on the $f_c.a$ axis), -0.42 kHz (on the f_c axis). Yeast (*Saccharomyces* sp.) protoplasts were prepared as in Ref. [21]. On-line in color.

As can be seen from Fig. 3, the low scatter of data on osmotically-swollen protoplasts indicates the regression of f_c on σ/a to be superior (r value of 0.9939 instead of 0.9859) to that of $f_c.a$ on σ. The membrane capacity values are very similar, but the intercepts are significantly different. However, as they are both negative, they are difficult to interpret.

It is notable that the yeast C_m values, at 0.61-0.64 µF/cm^2, are consistent or slightly below those of artificial lipid bilayer membranes [22]. This is unusual for cell membranes, where values of 0.8 µF/cm^2 or higher have been found in rotation work (except for some plant leaf cells where a large internal vacuole membrane is effectively in series with the plasma-membrane).

b) Cultured human T-cells ("DP7 cells")

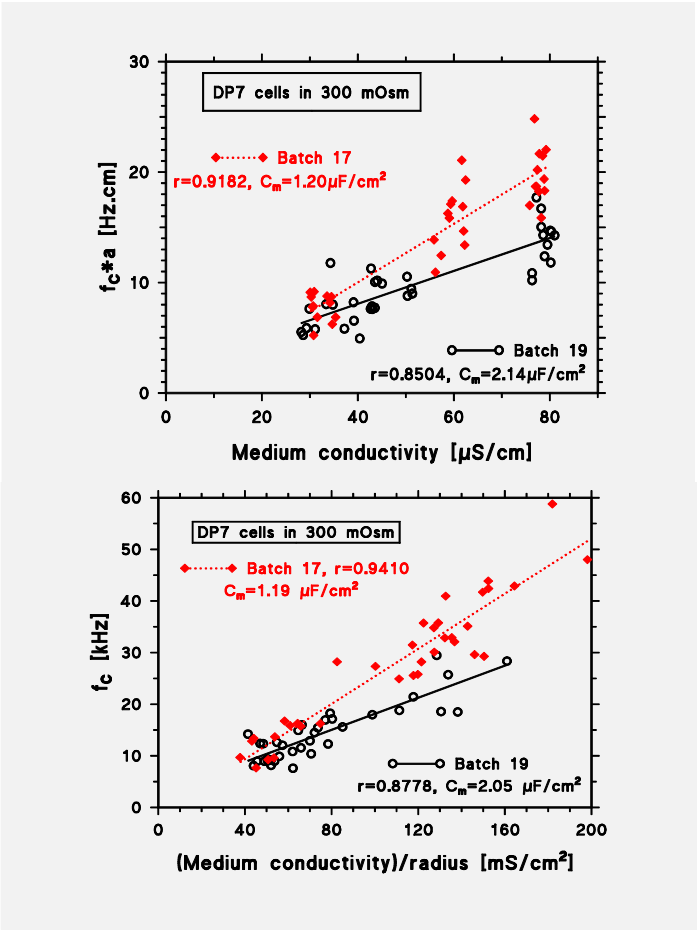

Fig. 4. Rotational measurements of two batches (36 cells each) of cells from a human T-cell line. The media contained 1 mg/ml serum abumin. The data are plotted separately using linear regressions according to Eq. 1 and Eq. 2, with comparison through correlation coefficients *r*. The intercepts were both negative for Batch 17 (-0.57 Hz.cm or -1.32 kHz), but both positive for Batch 19 (2.13 Hz.cm or 2.58 kHz). On-line version in color.

The DP7 cells exhibit relatively variable membrane capacities: the values of 1.1-2.1 μF/cm^2 reported here were the extremes of five batches measured on separate days. The majority appeared under the microscope to be covered with fine hairs, which could represent villi or other membrane ramifications. Immune-system cells, such as the T and B lymphocytes studied previously [10], show significant changes in membrane properties as they respond to stimulatory signals. It may be significant that the culture of the DP7 cells involves exposure to interleukin-2 and phytohaemaglutinin, on a weekly cycle.

For the DP7 data, regression of data plotted according to Eq. 2 yields regression coefficients that are significantly closer to unity (perfect linear correlation) than with Eq. 1.

c) Giant fused myeloma cells

These cells are formed from hundreds or thousands of cultured mouse myeloma cells by electrofusion. Their preparation, some rotational spectra, and a size distribution for the giant cells are described in another paper at this Conference [23].

Measured at physiological osmolality (300 mOsm), the scatter of the points above and below the regression lines is significant, and the regression coefficients are only moderately close to unity (perfect correlation). However, for these three batches of measurements, the regression coefficient using Eq. 2 is higher than that using Eq. 1.

In a strongly diluted solution (100 mOsm), the scatter is reduced, as reported before. Accordingly the correlation coefficients are closer to unity than in 300 mOsm, nevertheless use of a plot according to Eq. 2 has an advantage also in these three sets of data.

In all six cases, the membrane capacity values derived by use of Eq. 1 or Eq. 2 are very similar. The intercepts also appear to be correlated, although not very well.

IV. Discussion

Biological insight into cell structure and function can be derived from values of the membrane capacitance. Measurement of the anti-field rotation peak ($f_c.a$) on a statistically significant number of cells at a series of medium conductivities (σ) is a convenient way to gather sufficient data to perform a linear regression analysis between these two parameters, where the further variable cell radius (a) must also be included. Measurements on the same single cell at several different conductivities can avoid the problem of including a third variable in the regression analysis, but this is a difficult technique only suitable for larger cells (such as oocytes [9], or giant cells [23]).

For measurements on cell populations, it has been found here that regression of f_c values against σ/a invariably gives the same or a better correlation coefficient than regression of $f_c.a$ values against σ. Therefore use of the f_c versus σ/a method should improve the accuracy of the derived membrane data. This may be particularly significant where use of the intercept is made to derive a value for the membrane conductivity (and/or the surface conductance), because this intercept is small and the associated percentage error is larger than for the gradient.

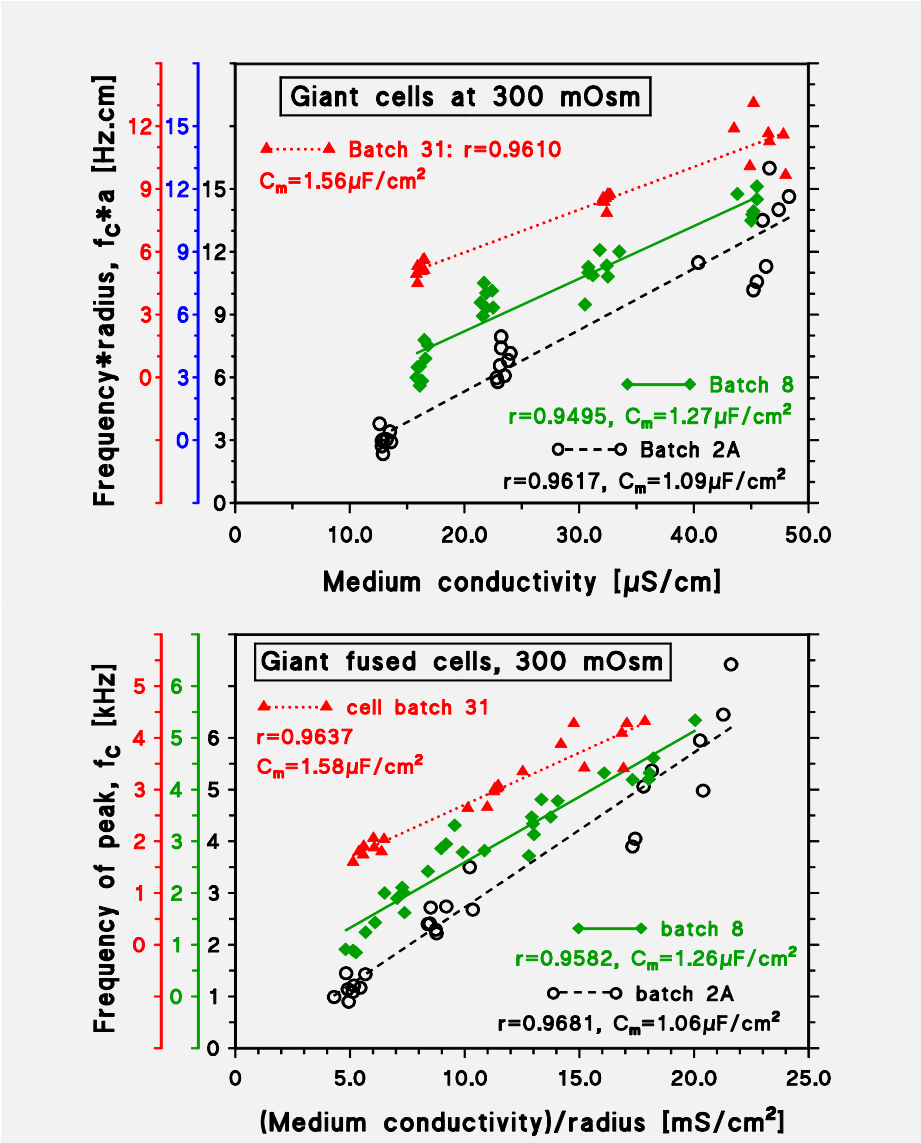

Fig. 5. Rotational measurements at 300 mOsm of three batches of electrofused giant SP2 cells plotted separately using linear regressions according to Eq. 1 and Eq. 2, with comparison through correlation coefficients *r*. A separate axis (and color, on-line) is used for each batch and its regression line. Intercepts were negative for Batch 2A (-0.53 Hz.cm and -0.27 kHz), but positive for Batch 8 (0.18 Hz.cm and 0.06 kHz) and for Batch 31 (1.88 Hz.cm and 0.70 kHz).

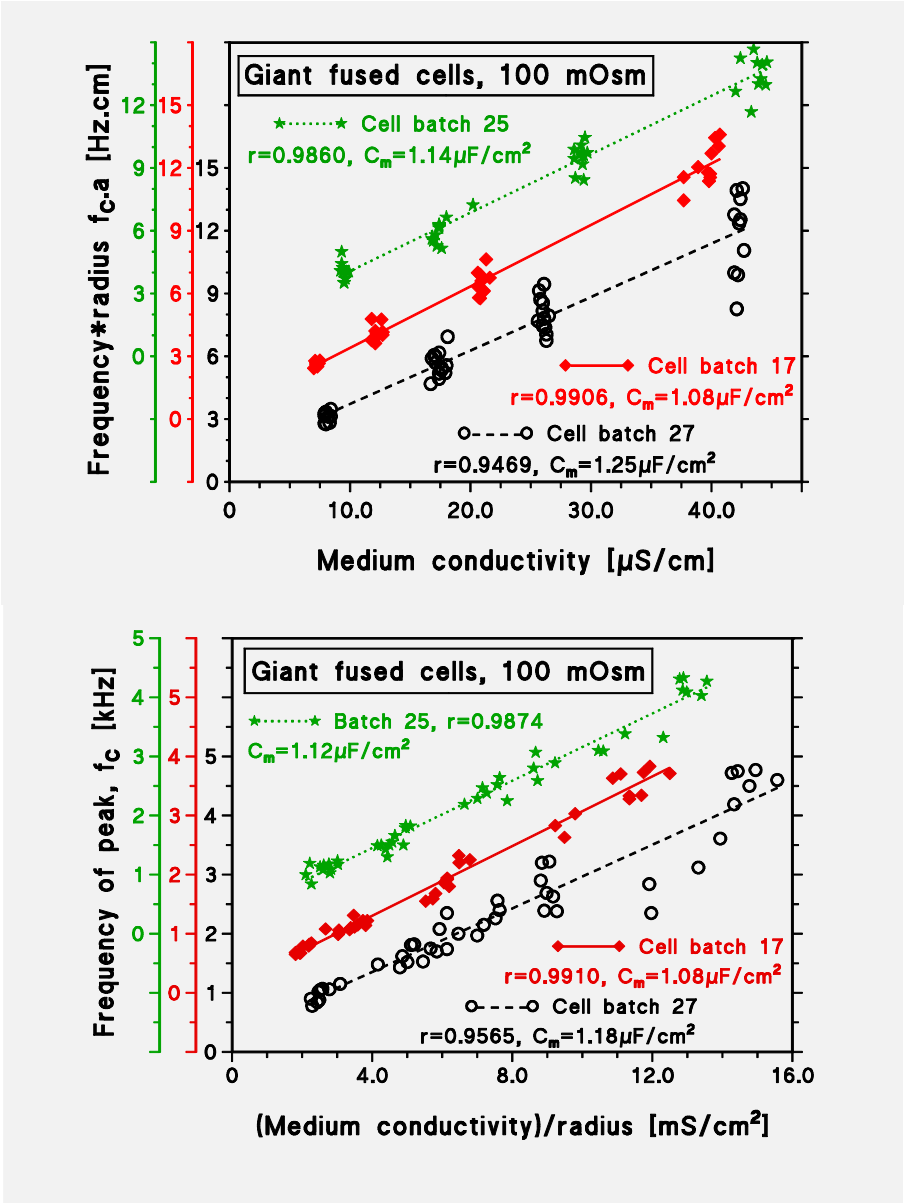

Fig. 6. Rotational measurements at 100 mOsm of three batches of electrofused giant SP2 cells plotted separately using linear regressions according to Eq. 1 and Eq. 2, with comparison through correlation coefficients *r*. A separate axis (and color, on-line) is used for each batch and its regression line. Intercepts were positive: for Batch 17 (0.46 Hz.cm and 0.12 kHz); for Batch 25 (1.28 Hz.cm and 0.31 kHz); and for Batch 27 (1.17 Hz.cm and 0.28 kHz).

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