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Determination of cell membrane passive electrical properties using frequency domain dielectric spectroscopy technique. A new approach

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To take into account the highly irregular surface morphology of cell membranes we have analyzed impedance measurements of biological cell suspensions in general terms using a fractal description of the surface roughness of the cell membrane. This analysis has been applied to human erythrocytes in different solutions of alkaline metal salts and to human lymphocytes, since these cells present a different surface irregularity. The passive electrical properties (dielectric constant and electrical conductivity) deduced from conductivity measurements at radiowave frequencies have been discussed on the basis of the fractal dimension of the membrane surface.

Impedance measurements represent a well-established technique of continuing importance for studying the passive electrical properties of biological cell membranes in aqueous suspensions and have been used in a great variety of systems of biological relevance [1].

The method, which has been widely reviewed in this context [2], takes advantage of the Maxwell-Wagner effect, occurring at the interface between the cytoplasm and the poorly conducting membrane, as a consequence of the different electrical properties (conductivity and dielectric constant) of the adjacent media.

From the most general point of view, the complex conductivity, σ^*, of a heterogeneous system consisting of shelled inclusions randomly dispersed in a continuous medium must be accounted for a relation of the form

$$\sigma^* = f(\sigma_m^*, \sigma_p^*, \sigma_s^*, \Phi, g_k) \quad (1)$$

where σ_m^*, σ_p^*, σ_s^* are the complex conductivities of the external medium, the cytosol and the membrane respectively, g_k represent parameters on the geometry of the dispersed phase and Φ is the fractional volume of the dispersed phase.

The dependence on frequency of the conductivity, σ^*, is taken into account considering the three phases involved in the dispersed system as characterized through the general expression

$$\sigma^* = \sigma + \iota\omega\varepsilon_0\varepsilon^*$$

where σ is the d.c. conductivity, ω the angular frequency of the applied field, ε_0 the permittivity of free space and ε^* the complex dielectric constant

$$\varepsilon^* = \varepsilon'(\omega) - i\,\varepsilon''(\omega)$$

including both the permittivity ε' and the dielectric loss ε''.

The models by which the function f can be obtained are most often based on the effective medium and mean-field theories. The functional dependence of σ^* on the parameters of Eqn. 1 has been originally given by Pauly and Schwan [3] for spherical particles and by Hanai and co-workers [4] for ellipsoidal particles.

In this technique, the overall dielectric constant and/or the conductivity of the cell suspensions are measured in the radiowave frequency range and based on these data, membrane properties (the conductivity σ_s and the dielectric constant ε_s) are extracted using the appropriate mixture equation by a nonlinear least-square curve-fitting procedure.

Recently, the present authors [5] have proposed an extension of the above analysis to take into account the main features of the membrane, i.e., the hydrocarbon chains and the polar group layers extending into the aqueous phase. The equivalent circuit adopted consists of three series resistor-capacitor elements which model the electrical behavior of the inner hydrophobic phase

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(through the capacitance C_H and the conductance G_H per unit surface) and the outer hydrophilic phases (through the capacitance C_p and the conductance G_p per unit surface) of the cell membrane.

The correlation of the passive electrical parameters with the functionality, the organization and the dynamics of biological membranes is an important area of study for physiological research.

Within the framework of the above model, the electrical behavior of the cell membrane is described by the two parameters, σ_s and ε_s which take into account the 'global' properties of the medium, or by the four parameters, G_H, G_p, C_H, C_p, if the different regions of the cell membrane are modeled by the equivalent resistor-capacitor circuit elements. In any case, this description is insensitive to the morphological details resulting in surface irregularities of the membrane.

On the other hand, biological cell membranes frequently exhibit numerous membrane protusions which tend to increase significantly the actual membrane area. This highly irregular (and perhaps fractal) surface morphology can have a pronounced effect on impedance measurements especially at time-scales corresponding to audiofrequency range in a.c. measurements. The effect of surface roughness on impedance measurements, using traditional models, can give rise to a significant overestimation of the membrane electrical parameters.

The fractal description of the surface may circumvent these difficulties, since fractals [6] offer an efficient way of characterizing irregularity in very general terms [7] and consequently the determination of the fractal dimension of the membrane surface could provide a more significant evaluation of the membrane passive electrical properties and a better understanding of different types of membrane–membrane interaction.

Recently, De Levie [8], using scaling arguments, has proposed that the interfacial admittance, Y, per unit 'apparent' interfacial area can be described over a wide range of frequency by the empirical relationship

$$Y = b\, y^{\alpha} \tag{2}$$

where the exponent α is related to the fractal dimension d_f of the surface by

$$\alpha = 1/(d_f - 1) \tag{3}$$

and y is the interfacial admittance taken per unit of 'true' microscopic area. For any two-terminal RC network the surface admittance is simple given by $y = G + \iota\, \omega\, C$ with C and G the capacity and the conductance of a small two-dimensional element of the membrane surface. As pointed out by Nyikos and Pajkossy [9], the value of α offers a simple comparison between surfaces with widely different morphologies and hence can be regarded as a measure of the surface roughness. The impedance behavior of rough surfaces is intermediate between that of planar ($\alpha = 1$; $d_f = 2$) and that of a porous ($\alpha = 1/2$; $d_f = 3$) surface.

Nyikos and Pajkossy [9] have derived an expression for the proportionality constant, b, in Eqn. 2 which can be generalized to a case of a membrane in the form

$$b = h(1/\sigma_m + 1/\sigma_p)^{\alpha - 1}$$

where σ_m and σ_p are the ionic conductivities of the bulk solution and cytosol, respectively, and h is a purely geometrical factor.

In this way, the originally two parameters (ε_s and σ_s) or the four parameters (G_H, G_p, C_H, C_p) give rise to the four parameters h, α, C, G which allow the full description of the electrical behavior of the cell membrane through the expressions

$$\varepsilon_s = \varepsilon_0/d\ \mathrm{Re}\{Y\}$$

$$\sigma_s = 1/d\ \mathrm{Im}\{Y\} \tag{4}$$

where d is the 'apparent' membrane thickness. Whereas the parameters C and G are related to the structural features of the membrane, the geometric factor h reflects the fact that the fractal description ignores the details of the surface morphology.

This analysis has been applied to biological cells of different surface irregularity: human erythrocytes in different solutions of alkaline metal salts and human lymphocytes, whose surface [10], due to the existence of many microvillous projections, is highly rough.

Erythrocytes were obtained from blood of normal donors. The plasma and buffy coat were removed and the red cells washed twice in isotonic saline solution. The erytrocytes were then resuspended, to make a hematocrit of 30%, in various electrolyte solutions of different alkaline metal salts (NaCl, CsCl, LiCl, KCl) at a concentration of 0.15 M and were incubated at 25 °C so as to attain an ionic equilibrium of saline solution across the membrane. The conductivity measurements carried out in the frequency range from 10 kHz to 100 MHz have been reported in detail elsewhere [11].

Human lymphocytes were isolated from human blood. The cells were harvested by centrifugation. The supernatant was removed and the cells were resuspended in 0.15 M NaCl saline solution to a final concentration of about 10^6 cells per μl. Lymphocyte suspensions contain both the B and T cells. The full account of these conductivity measurements will be published as a separate paper elsewhere in the near future.

Figs. 1 and 2 show the measured conductivity of erythrocyte and lymphocyte suspensions respectively as a function of frequency. The most relevant results of this analysis are summarized in Table I.

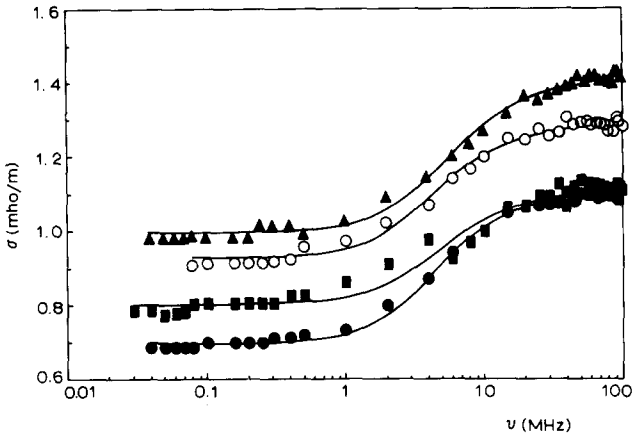

Fig. 1. Conductivity of erythrocyte suspensions in different 0.15 M electrolyte solutions as a function of frequency. ▲, KCl; ○, CoCl; ■, NaCl; ●, LiCl. The temperature is 25 °C. The hematocrit is $\Phi = 0.30$. The solid curves represent the best-fit dispersion curves calculated with the parameters listed in Table 1.

TABLE I

Some electrical parameters of erythrocytes and lymphocytes determined by fitting Eqn. 4 to the conductivity dispersion curves shown in Figs. 1 and 2

Cell suspensions	Electrolyte solution 0.15 M	C (F/m^2)	G (Ω^{-1}/m^2)	σ_p/σ_m	d_f
Erythrocytes [a]	NaCl	$3.17 \cdot 10^{-3}$	$9.10 \cdot 10^3$	0.43	2.01
$\phi = 0.30$	KCl	$1.79 \cdot 10^{-3}$	$1.87 \cdot 10^3$	0.43	2.00
	CsCl	$1.75 \cdot 10^{-3}$	$2.30 \cdot 10^3$	0.36	1.99
	LiCl	$3.55 \cdot 10^{-3}$	$6.77 \cdot 10^3$	0.72	2.00
Lymphocytes [b]					
$\phi = 0.64$	NaCl	$4.10 \cdot 10^{-4}$	$1.04 \cdot 10^6$	0.77	2.19

[a] The erythrocyte is simulated with an oblate spheroid of 8 μm in major diameter and 2.4 μm in minor diameter. The thickness of the membrane is assumed to be 50 Å.

[b] The lymphocytes were almost spherical with a mean radius of 5.64 μm. The size distribution based on microscopic observations results in a standard deviation of about 0.2 μm.

The membrane electrical parameters of erythrocytes are almost consistent with those reported so far [5]. The membrane capacitance, C, in the case of NaCl solution is in reasonable agreement with that ($C = 6.8 \cdot 10^{-3}$ F/m^2) measured by Takashima et al. [12]. This value agrees closely with the value of $4.3 \cdot 10^{-3}$ F/m^2 derived from those of hydrophilic ($C_p = 1.1 \cdot 10^{-3}$ F/m^2) and hydrophobic ($C_H = 1.8 \cdot 10^{-3}$ F/m^2) regions of the membrane previously estimated, on the basis of the lumped RC network.

It must be noted, however, that in the present case the estimated capacitance and conductance concern with the 'true' microscopic surface of the cell membrane and, in principle, they may differ from those describing the average property of the membrane.

The effects of different cations on the structure and the permeation of erythrocyte membrane discussed on the basis of our membrane electrical model seems to be confirmed by the present analysis. Moreover, the conductivities σ_s of the internal medium are in good agreement for all the saline solutions with those previously reported [5]. As can be seen in Table I, it is noteworthy that the fractal dimension of the erythrocyte surface does not differ appreciably from 2 and is consistent with a relatively smooth surface, indicating that the membrane structure uniformly fills a two-dimensional space.

The overall agreement between the parameters of the present analysis and those deduced from a single-shell model which considers the membrane as a homogeneous medium and the cell interior as a homogeneous phase, provides a further support to the value $d_f = 2$.

The lymphocyte membrane provides an interesting contrast. In fact, it is noteworthy that in this case the fractal dimension assumes a value of about $d_f = 2.2$ reflecting the existence of a more complex surface cell morphology due to the presence of extended microvillous structures which characterize the cell-surface architecture.

Moreover, in this case we obtain for the plasma membrane capacitance a value of about $4 \cdot 10^{-4}$ F/m^2 and for the membrane conductance a value of the order of 10^6 ohm^{-1}/m^2. These values differ greatly from those estimated by more conventional analysis. It must be noted, however, that in spite of the importance of lymphocyte membrane in immunological functions, very few dielectric and conductometric data on these cells are, at present, available.

Recently, Surowiec et al. [13] have estimated the membrane capacitance of B and T lymphocytes on the basis of approximate relations derived by Schwan [14] obtaining values of $2.9 \cdot 10^{-2}$ F/m^2 and $7.7 \cdot 10^{-3}$ F/m^2, respectively. More recently, Asami et al. [15],

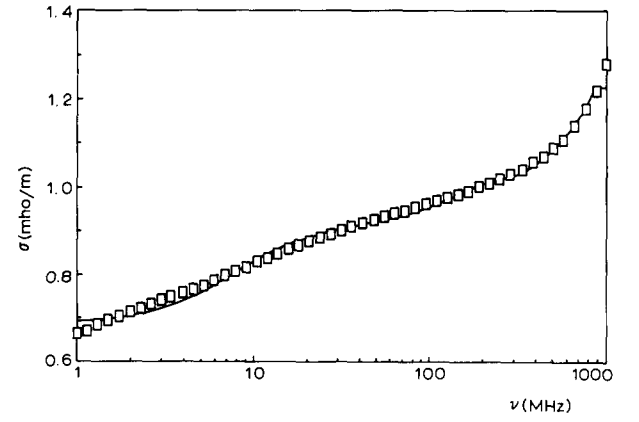

Fig. 2. Conductivity of lymphocyte suspension in physiological saline solution as a function of frequency. The temperature is 20 °C. The fractional value is $\Phi = 0.64$. The solid curve represents the best-fit dispersion curve calculated with the parameters listed in Table I.

using a double-shell model [16,17] have reported, in the case of mouse lymphocytes, values of the capacitance of the plasma membrane of $8 \cdot 10^{-3}$ F/m^2. On the basis of the assumption that $\sigma_s/\sigma_p = 10^{-5}$, the conductance of the plasma membrane can be valued to be of the order of $0.4 \cdot 10^3$ ohm^{-1}/m^2.

The high value of the membrane capacitance and conductance deduced from the present analysis may be roughly explicable considering that the microvillous structure allows an higher ionic permeation with an increase of the ionic hydration. Another finding supporting the present results is that if the apparent thickness of the plasma membrane is considered as a free parameter in the model, the best fit was obtained with $d = 0.85$ μm, which is much greater than can be accounted for by microscopical inspection. In other words, the fractal-like properties of rough interface extend over a range of length of the order of 1 μm.

It must be noted, however, that erythrocytes and lymphocytes deeply differ in their intracellular structure. Whereas erythrocyte does not possess nucleus and cytoplasmic organellae, lymphocyte has a nucleus occupying a large fraction of the total cell volume. For both the cells, the above analysis is based on a single-shell model, since we are mainly interested in the electrical properties of the outer membrane and in the characterization of its fractal dimension. A more appropriate model, for example the double-shell model proposed by Irimajiri [16,17] might provide a better description of the lymphocyte dispersion [15] and consequently of the membrane passive electrical properties.

Althought preliminary, these results demonstrate the utility of the fractal description of the surface roughness of the cell membranes. Cell surface characterization is of fundamental importance in many events and the above analysis can give insight into the structural arrangement at the membrane surface or into the changes also occurring with both growth stimulation and malignant transformation.

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