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DIFFERENTIAL DIELECTRIC BEHAVIOR OF THE PLASMA MEMBRANE IN MOUSE FIBROBLASTS AND HUMAN EMBRYO KIDNEY CELLS.

Biological Science

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ABSTRACT

The dielectric features of "native" human embryo kidney cells (HEK293) and mouse fibroblasts (3T6) were investigated by electrorotation. The specific dielectric parameters Capacitance (C) and Conductance (G) exhibit a significant difference between the two cell lines. In the case of the human one, both parameters result in an increase by one order of magnitude, in the early stage of the culture. This drastic difference with respect to 3T6 cells, tends to disappear as the culture time proceeds. Parameter C is directly related to the membrane structure, while G mainly derives from the active and passive ion flux. The variation of these parameters is discussed in terms of an intrinsic functional/structural difference of the cell membrane in the two cell lines. Finally, the phenomenon is not ascribable to membrane mal-functions, as evidenced by cellular/molecular assays.

KEYWORDS

Electrorotation, Membrane Dielectric Properties, HEK 293 cells.

1. Introduction

The cytoplasmic membrane allows the transport of substances and, in particular ions, from the surrounding environment to the cytoplasm matrix: this transport can be passive or active. In this latter case, the cell expends energy in the process. In addition, the membrane is endowed of an intrinsic electrical potential, therefore, the biological membranes participate to passive osmosis and diffusion as well as transmembrane protein channels (Alberts et al. 2012).

The membrane integrity and functionality play a fundamental role in a number of cell functions and they are essential for the maintenance of the cell's physical shape, texture and solidity. Therefore, the efficient functioning of the cell membrane is essential for cell survival. With respect to this, electrorotation (ER) represents a well-established tool giving a significant information about the intra-membrane traffic and structural properties of the membrane (Bonincontro and Risuleo 2015). This technique permits, in fact, measuring two essential biophysical parameters of the cell membrane (specific capacitance C and specific conductance G, where the term specific means "per surface unit"): these parameters are strictly linked to the structure/function relationships of the bio-membrane. In addition, one of the major advantages of ER consists of the possibility to observe and measure rotating single cells in an electric field (Mansor and Ahmad 2015).

In this work we present an investigation of two particularly different cell populations as far as their cytological origin is concerning. One line is represented by mouse fibroblasts (3T6) which was extensively investigated in our laboratory and therefore is considered like a standard of reference for the above mentioned membrane parameters; the second one is a human kidney embryonal cell line (HEK 293). These kidney cells are widely used in cell biology due to their reliable growth mode and propensity for transfection. In addition, they are used in biotechnology to produce potentially therapeutic agents for gene therapy. Due to their intrinsic cellular, physiological and molecular properties, a comparative study of their electrorotational behavior, becomes of primary interest. Both cell lines were studied at two different culture times, which may influence the metabolic and therefore, the structural characteristics of the cell. In particular, the definition of their membrane biophysical properties may, in the long run, allow the optimization of transfection protocols (Cosimati et al. 2013; Stefanutti et al. 2014; Majzoub et al. 2016; Stewart et al. 2016).

2. Materials and Methods

2.1 Cell Cultures

The stable murine fibroblasts 3T6 and HEK 293 lines were used throughout the work. The cultures were maintained according to routine procedures in DMEM-10% newborn serum supplemented with glutamine and penicillin-streptomycin. Cells were plated and growth was continued for 24 or 48 hours; they were washed and resuspended in an osmolar sucrose solution which rules out the possibility of osmotic stress. The same solution was supplemented with low NaCl concentrations to vary the conductivity of the dispersing buffer. Electrorotation measurements were immediately performed (Milardi et al. 2014; Muzi et al. 2016).

Vital cell count was carried out by trypan blue exclusion according to standard procedures. Cell counts were done in a Burker chamber and the cells in 12 different sectors were counted.

The cell cycle distribution of the HEK 293 subpopulations was evaluated, on non- fixed cell samples, by cytofluorimetric analysis. Prior to the analysis cells were washed twice with ice-cold PBS, kept on ice and immediately analyzed (for further details see Milardi et al. 2014).

2.2 Electrorotation Theory

A rotating electric field, applied to cell suspensions, induces on each single cell an effective dipole moment deriving from the different polarizability between solvent and plasma membrane. If the mechanism of interfacial polarization is in phase with the electric field, the induced dipole moment is aligned. As the frequency of the field increases, the polarization mechanism undergoes a phase delay (dielectric relaxation) generating a torque moment and the cells rotate in an anti-field fashion. This relaxation occurs in the frequency range of 104 - 106 Hz and is directly related to the dielectric properties of the plasma membrane (Foster et al. 1992; Gimsa 2001). The rotation period (T) of the cell depends on the frequency (f) of the applied field, according to equation (1), which describes a Debye-like relaxation due to a Maxwell-Wagner effect:

$$T(f) = T_{\min} \frac{1+(\frac{f}{f*})^2}{2(\frac{f}{f*})} \qquad (1)$$

where, f* is the relaxation frequency and Tmin is the corresponding value of the cell rotational period . The experimental data must be analyzed by fitting according to equation (1) to obtain the relaxation frequency. The value of f* depends on the solvent conductivity according to the equation (2):

$$f^*=\left(\frac{1}{\pi R \quad C}\right)\sigma_e+\left(\frac{1}{2\pi \quad C}\right)G \qquad (2)$$

where C and G are, respectively, the specific capacitance and specific conductance of the plasma membrane of a cell with a radius R; e is the solvent conductivity. Equation 2 is valid if e is negligible with respect to the conductivity of the cytoplasm Gimsa, 2001; Mansor and Ahmad, 2015 and references therein). Furthermore, the light-microscope observation allows also an accurate estimate of the average cell radius.

The relaxation frequency is measured at different conductivities of the cell-dispersing buffer, taking into consideration the average estimated cell radius. Typically, four dispersing solutions are used: sucrose 300 mM and supplemented with NaCl at three different low concentrations (Bonincontro and Risuleo 2015). To have a good estimation of the absolute value of C and G, and R a statistically reasonable number of cells was monitored.

The electrorotation apparatus and the measuring set up were previously described in great detail (Cosimati et al. 2013; Bonincontro et al. 2007; Bonincontro and Risuleo, 2015).

4. Results and Discussion

The Debye model envisages the cell as a sphere surrounded by a thin homogeneous layer that simulates the plasma membrane. This simplified model is commonly accepted as a good tool to investigate the features of the biological membrane. In comparison to the complexity of the real biological system, this model is rather coarse; however, it proved very effective to evaluate alterations in the dielectric properties of the cell membrane. The capacitance C depends upon the biochemical and physical properties of the membrane i.e.: the lipid moiety and the presence of structural/functional polypeptides as well as their assembly and interactions. The conductance G, on the other hand, is informative of the membrane activity: in this case, the membrane is considered as the interface between the cell matrix and the outer environment in terms of metabolism/ion-transport. Figure 1 (A and B, HEK 293 cells) and 2 (A and B, 3T6 cells) report the linear fits of the relaxation frequencies as a function of the conductivity of the dispersing medium. Following the application of the Debye model, the values of C and G are obtained. Table 1 summarizes the results of the electrorotational measurements for both culture times and cell lines. The differences of the dielectric parameters in the HEK 293 cells are dramatic with respect to the 3T6: moreover, they are strongly dependent upon the culture time. In particular, at 24 hours, HEK293 cells exhibit C and G values one order of magnitude greater than those shown by the mouse fibroblast line. However, after 48 hours of culture time, we observed that, while 3T6 cells retain the same electro-rotational values, HEK 293 cells show a remarkable drop of both C and G, accompanied by a significant increase of the cell radius.

Data obtained by other Authors on HEK293 cells at 72 hours of culture time, show C and G values comparable to those monitored by us on 3T6 cells at shorter culture times. Notably, these electrorotational parameters underwent in our experimental measurements, a drastic drop already at 48 hours of culture time. In addition, we measure a cell radius with an increasing trend. Therefore, the discrepancy is only apparent and the literature data at 72 hours of culture time are consistent with our observations (Zimmermann et al. 2008a; Zimmermann et al. 2008b; El-Gaddar et al. 2013).

It is worth noting that these variations are not paralleled by a relevant cell death as monitored by viability tests consisting of vital dye exclusion (trypan blue; as reported in Table 2). These results imply that the overall condition of the cell is not affected by the culturing time but, on the contrary, the alterations of the dielectric parameters are attributable to the permanence in culture. Moreover, the overall cell metabolism does not seem to be particularly affected as shown by the cytofluorimetric analysis of the cell cycle (Milardi et al., 2014). As a matter of fact, HEK 293 do not show significant variations of the distribution of the cell sub-populations in the various stages of the cell cycle at 24 and 48 hours of culture time (Figure 3). The small percent variation of the three cell subpopulations is not significant and, since cells are not synchronous, this is very likely due to the intrinsic dis-homogeneity of the sample. This result, although indirectly, demonstrates that the permanence in culture does not have negative consequences for the overall cell function.

It should be born in mind that biological tests take into account highly complex phenomena regarding the whole cell function and vitality. On the other hand, ER analyzes exclusively the plasma membrane and, in particular, the distribution of the electric charges at protein level. Such phenomena may have a transitory duration during cell growth and this transient character was already observed in our laboratory in different experimental conditions aimed at the assessment of the membrane permeability (Berardi et al. 2009a; Berardi et al. 2009b; Cosimati et al. 2013; Milardi et al. 2014). However, under the biophysical point of view, the electrorotational data may be interpreted in alternative but not mutually exclusive terms.

In fact, as mentioned above when the Debye single-shell model was discussed, the physical surface of the plasma membrane is greater than an ideal sphere due to the presence of microvilli and invaginations. Therefore, a form factor needs to be introduced in the equation of the specific capacitance of the spherical condenser associated to the cell:

C = Km 0 ’/d

Where, d is the average membrane thickness, ’ is the membrane permittivity, 0 is the vacuum permittivity and Km is a dimensionless coefficient higher than 1 [see for instance the classical treatment reported in Cosimati et al. 2013]. Therefore, one may hypothesize that HEK293 cells, at early stages of culture, have a more extended "effective" surface. In fact, a recent study showed that the rigidity and mechanical properties of these cells vary when they are in suspension or in an adhering status. This phenomenon has been attributed to the alterations of the cytoskeleton and in particular to the action of actin (Hagparast et al. 2015). A second possible explanation is related to the inner structure of the membrane bilayer. The capacitance of this latter is directly proportional to the permittivity ’. It should be pointed out that the proteins mainly influence the membrane permittivity (Singer and Nicholson 1972). Indeed, this parameter in the case of a lipid double layer should be of the order of two units (Pethig and Kell 1987), while in general it is 4-12 units for the cell membrane (Asami 1977; Hanai et al. 1979; Bonincontro and Mariutti 1988; Polevaya et al. 1999). An augmentation of ’ implies a higher concentration of proteins which are more regularly arranged. In fact, an irregular distribution of polypeptides would not give a significant contribution to the polarization. As the culturing time proceeds, the biological effect of what just discussed, consists of a gradual and partial de-structuration of the plasma membrane, which is accompanied by an increase of the overall cell diameter. Finally, as a third possibility, a reduced membrane thickness may contribute to the effect on the dielectric parameters, but the contribution of this thickness reduction are in general hardly assessable. It is known, as a matter of fact, that the variations of membrane thickness are in the order of some nanometer decimals (Alberts et al. 2012).

As far as G is concerning (G = /d where is the membrane conductivity and d its thickness) the structural phenomena proposed for C, can influence the same trend of the membrane specific conductance, which in vital cells is principally attributable to an active ion transport mediated by membrane-protein interactions (Bonincontro and Risuleo 2015).

5. Conclusions

The major effect on the Human Embryonal Kidney cells (HEK293) consists of very high values of C and G after a relatively short culturing time. As the cell culture is prolonged, these parameters diminish and tend to reach the values reported in literature at 72 hours. These values are comparable to those shown by the 3T6 fibroblast cells. Three different interpretations may be involved in the phenomenon: texture of the outer cell surface, permittivity, which is a specific feature of the membrane structure and membrane thickness. The data amply discussed above convinced us that the variation of ’, which is directly related to the membrane structure may be the prevailing cause of the experimental observations.

Cell samples	*C* (F/cm^2)	*G* (S/cm^2)	R ( )
HEK 293			
Culture time(24 h)	15 ± 2	10 ± 2	5.3 ± 0.2
Culture time (48 h)	5 ± 1	3.1 ± 0.7	6.2 ± 0.2

3T6			
Culture time (24 h)	0.9 ± 0.2	0.27± 0.05	9.7 ± 0.2
Culture time (48 h)	1.0 ± 0.2	0.29± 0.05	9.9 ± 0.2

Table 1. Comparative summary of the membrane dielectric parameters *C* (specific capacitance), *G* (specific conductance) and R (average cell radius) for HEK 293 and 3T6 cells. Culturing time 24 and 48 hours.

Cell samples	Total cell number	Survival (%)	Death (%)
HEK 293			
Culture time (24 h)	354	76	24
Culture time (48 h)	339	90	10
3T6			
Culture time (24 h)	271	96	4
Culture time (48 h)	404	91	9

Table 2. Survival and death rate for 3T6 and HEK 293 cells, reported for a culture of 24 and 48 hours as indicated in the Table. The cell number in each considered sector varied by a few percent.

Figure 1

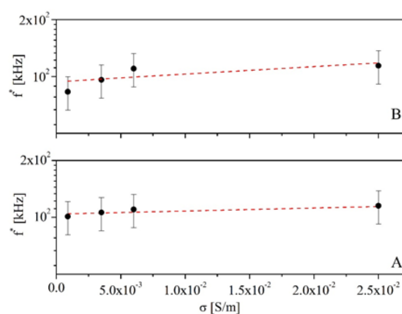


Figure 2

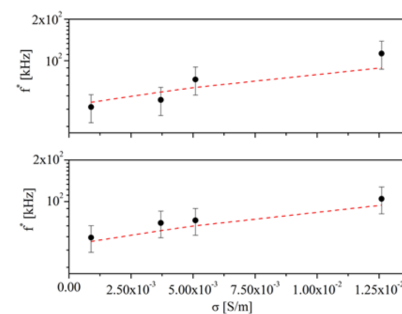
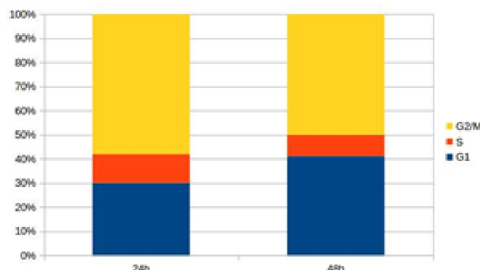


Figure 3



Captions to the Figures

Figure 1: Linear fit of the relaxation frequency as a function of the dispersion buffer referred to the HEK 293 cells. (A) and (B) Culturing time was 24 and 48 hours, respectively.

Figure 2: Linear fit of the relaxation frequency as a function of the dispersion buffer referred to the 3T6 cells. (A) and (B) Culturing time was 24 and 48 hours, respectively.

Figure 3: Cytofluorimetric analysis of the distribution of the cell subpopulations in the various phases of the cycle (Gap 2/Mitosis; S: DNA synthesis; G1: Gap 1). Analysis was done both at 24 and 48 hours of culture time. The percent of each cell subpopulation varied within 5,5%.

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