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Cell-based screening for membranal and cytoplasmatic markers using dielectric spectroscopy

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ABSTRACT

Dielectric spectroscopy (DS) of living biological cells is based on the analysis of the complex dielectric permittivity of cells suspended in a physiological medium. It provides knowledge on the polarization–relaxation response of cells to external electric field as function of the excitation frequency. This response is strongly affected by both structural and molecular properties of cells and therefore, can reveal rare insights on cell physiology and behaviour. This study demonstrates the mapping potential of DS after cytoplasmatic and membranal markers for cell-based screening analysis. The effect of membrane permittivity and cytoplasm conductivity was examined using tagged *MBA* and *MDCK* cell lines respectively. Comparing the permittivity spectra of tagged and native cell lines reveals clear differences between the analyzed suspensions. In addition, differences on the matching dielectric properties of cells were obtained. Those findings support the high distinction resolution and sensitivity of DS after fine molecular and cellular changes, and hence, highlight the high potential of DS as non invasive screening tool in cell biology research.

1. Introduction

The dielectric properties of biological cells can provide rare insight about the cellular and molecular state of given cells. Dielectric study of biological cells was explored for the first time by Hoeber, who investigated the dielectric properties of *Erythrocytes* [1]. Here the cells were characterized as poorly conducting membrane enclosing a conducting and polar cytoplasm. The foundations of understanding the dispersion mechanism of biological cells suspended in physiological medium have been suggested by Schwan according to the theories of mixed dielectrics, founded by *Maxwell & Wagner* [2,3]. Since then, the dielectric properties of many cell types have been explored by a number of authors [4–7].

Dielectric properties of biological cells are mainly characterized by β dispersion mechanism which accounted for the medium (MF) and high (HF) range of the radio spectrum [7,8]. This mechanism is due to *Maxwell–Wagner* effect (interfacial polarization) at the external and internal interfaces of the phospholipids membrane [9,10]. In addition, contribution may also arise from dispersion of small intra cellular organelles, which usually appear as small adjunct to the β dispersion at high frequencies.

Dielectric spectroscopy (DS) techniques can provide comprehensive knowledge about cell structure and properties, intra cellular content, membrane shape and selectivity and many more [11–13]. Using dynamic dielectric spectroscopy allows real time quantification of cellular and molecular processes occurring in cells during variety of biological mechanisms like: growth, activity, division etc. [14–18].

Recent interests in the development of new high throughput, non destructive screening techniques are largely driven by the potential for associating physical methods with cell and molecular biology methodologies. Cell-based screening is a powerful method that uses living cells to test the effect of different molecules like drugs and toxicants on the cellular and molecular phenotype of cells. This technique requires the use of high sensitive tools that permit high speed systematic identification of biochemical targets and markers on given cell libraries.

This study is aiming to demonstrate the use of DS as potential screening tool after bio-markers in cell-based screening analysis. This approach can offer label/mediator-free strategy for rapid analysis and monitoring of cellular and molecular markers in living cells. It can provide fast and reliable prediction on the viability and physiology of cells as response to induced chemical stimulations or can be used to monitor development and transformation processes occurring in the cell within its life cycle. In order to understand the practicable impact of it, it is first essential to understand the more fundamental research of membranal and cytoplasmatic tagging effect.

Madin-Darby Canine Kidney Epithelial (MDCK) and *Bone Marrow derived Pre-Osteoblastic (MBA)* cell lines were used to investigate the

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effectiveness of DS to follow intra cellular and membranal markers. The intra-cellular marker used was based on *SEC13* protein over-expressing *MDCK* cells. Effect of membrane was tested in *lectin*-labeled *MBA* cells that bind glycosylate elements on cell surface. The analyzed dielectric spectra confirm the modulation of both markers on the dielectric parameters of cells. Cytoplasm conductivity of *SEC13* over expressed and membrane permittivity of *MBA* coated cells were found to be affected when compared to the native cell lines. Those findings provide the necessary knowledge regarding the ability to correlate between molecular and cellular markers, and changes on the matching complex permittivity spectra and parameters of the cells.

2. Modeling the dielectric response of cells

Analysis of dielectric parameters depends on cell topography; therefore, a matched electric model should be applied based on those geometrical characteristics. Most biological cells can be treated as thin insulating shelled component (membrane) enclosing conducting medium (cytoplasm). This simple description brought many theories and models developed by several authors. The first model of shelled-spheroid was introduced by Frick [19], later it was extended to the case of shelled-ellipsoid particles [20]. Those models however, were limited to low frequencies analysis. Since then, several models were proposed by Pauly and Schwan [21], Hanai et al. [22], and Redwood et al. [23]. The first comprehensive model was introduced by Asami and Hanai, who for the first time presented unlimited approach for dielectric analysis of suspended shelled-ellipsoids [24].

Both *MBA* and *MDCK* cells exhibit an approximate elliptic structure (Fig. 1), and therefore, can be treated using the dispersed shelled-ellipsoids analysis. Here the cells are described as two phase systems (membrane and cytoplasm) surrounded by infinite and continues medium (Fig. 2). Based on the theory of interfacial polarization, the complex relative permittivity of dispersed shelled-ellipsoids ε^*, suspended in a medium with complex relative permittivity ε_m^*, is given by:

$$\varepsilon^* = \varepsilon_m^* + \frac{\Phi}{3}\sum_{k=x,y,z}\frac{\bar{\varepsilon}_m^*\left(\varepsilon_{ck}^* - \varepsilon_m^*\right)}{\bar{\varepsilon}_m^* + \left(\varepsilon_{ck}^* - \bar{\varepsilon}_m^*\right)L_k} \tag{1}$$

Where $\Phi = \frac{4\pi R_x R_y R_z N}{3}$ is the volume fraction of the cells and ε_{ck}^* is the complex relative permittivity of the shelled-ellipsoid across the k axis, given by:

$$\varepsilon_{ck}^* = \varepsilon_{om}^*\left(1 + \frac{v\left(\varepsilon_i^* - \varepsilon_{om}^*\right)}{\varepsilon_{om}^* + \left(\varepsilon_i^* - \varepsilon_{om}^*\right)L_{ik} - \left(\varepsilon_i^* - \varepsilon_{om}^*\right)vL_k}\right) \tag{2}$$

Where ε_{om}^*, ε_i^* are the complex relative permittivities of the membrane and cytoplasm respectively and v is the volume ratio given by:

$$v = \frac{R_{ix}\cdot R_{iy}\cdot R_{iz}}{\left(R_{ix} + \Delta_L\right)\left(R_{iy} + \Delta_L\right)\left(R_{iz} + \Delta_L\right)} \tag{3}$$

Δ_L is the membrane thickness and L_k, L_{ik} (k=x, y, z) are the depolarization factors, given by:

$$L_k = \frac{R_x R_y R_z}{2}\int_0^\infty \frac{ds}{\left(R_k^2 + s\right)\sqrt{\left(R_x^2 + s\right)\left(R_y^2 + s\right)\left(R_z^2 + s\right)}} \tag{4}$$

$$L_{ik} = \frac{R_{ix} R_{iy} R_{iz}}{2}\int_0^\infty \frac{ds}{\left(R_{ik}^2 + s\right)\sqrt{\left(R_{ix}^2 + s\right)\left(R_{iy}^2 + s\right)\left(R_{iz}^2 + s\right)}} \tag{5}$$

Where R_x, R_y, R_z and R_{ix}, R_{iy}, R_{iz} are the semi-axes of the cell and cytoplasm respectively.

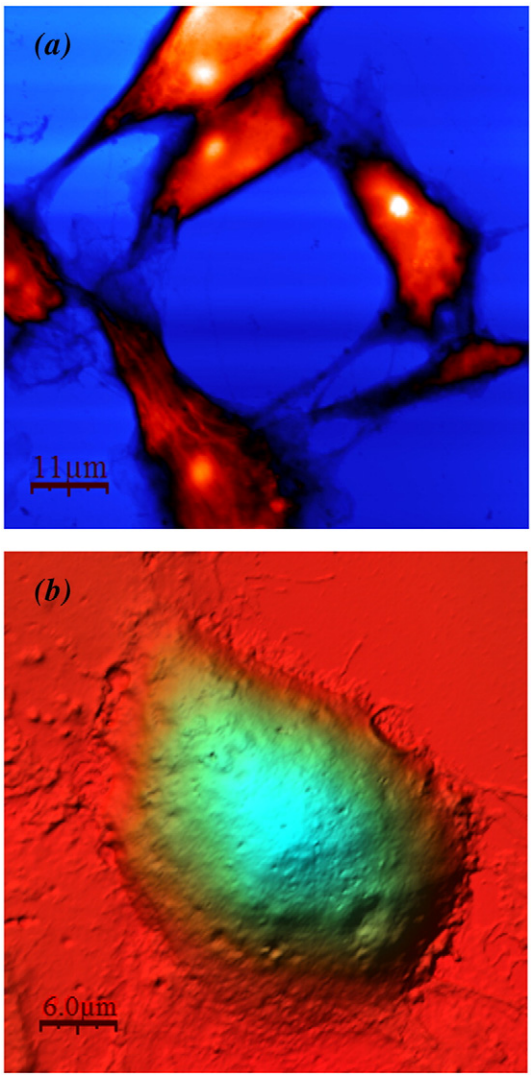

Fig. 1. Topographic images of cells. (a) Topography of joined *MBA* cells (70 μm×70 μm). (b) Topography of single *MDCK* cell (40 μm×40 μm). Both images recorded in physiological conditions in DMEM solution.

The medium relative permittivity is represented by the average value $\bar{\varepsilon}_m^*$. It can be found within the range of $\varepsilon_m^* \le \bar{\varepsilon}_m^* \le \varepsilon^*$, when it depends on cells concentration. At low concentration, the medium around each cell is assumed to be homogenous (cell free), and therefore, $\bar{\varepsilon}_m^* = \varepsilon_m^*$. When the cells concentration is high, each single cell surrounded by a mixture of cells and medium, and therefore, $\bar{\varepsilon}_m^* \approx \varepsilon^*$.

3. Methods

3.1. Preparation and analysis of cells

3.1.1. Cell cultures

Madin–Darby Canine Kidney Epithelial (*MDCK*) wild type or stable transfected with *SEC13*-GFP [25]. *MBA-15* cells are marrow stroma derived osteoprogenitors [26]. Cells were cultured in growth medium Dulbecco's Modified Essential Medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% glutamine and 1% antibiotics, and maintained in 5% CO_2 at 37 °C.

3.1.2. Fluorescence-activated cell sorter (FACS)

Binding of *biotinylated-lectin Helix pomatia* (*HPA*, Sigma) to cell surface carbohydrate residues expression were analyzed by FACS. For