Sensing and Bio-Sensing Research 21 (2018) 7–16

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Theoretical assessment of single-frequency electrical sensors for continuous monitoring of cell lysis in dilute suspensions

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ARTICLE INFO

Keywords:
Electrical sensing
Biological-cell suspensions
Lysis
Resistance
Capacitance
Effective dielectric function

ABSTRACT

We analyze the sensitivity of electrical measurements to lysis of biological cells in suspensions. We aim at discerning the electrical parameter and measuring conditions that are more convenient to monitor the kinetics of a lysis process. We consider single-frequency measurements of the capacitance and resistance of an electrical capacitor containing a suspension of biological cells at mid-radiofrequencies. We use well-established models to estimate the relative sensitivities to variations of the volume fraction of cells during a lysis process. Then we estimate values for the resolution in measuring the fraction of cells that have been lysed with commercially available LCR meters. Unexpectedly, our results indicate that, despite the total number of ions in solution remains constant during a lysis process, in general, the electrical resistance provides a more appropriate signal for sensing the evolution of cell lysis than the capacitance. This is contrary to the case when the cell's volume fraction without lysis is to be measured. We predict that, with single-frequency resistance measurements, a resolution better than 0.1% in volume fraction of lysed cells should be attainable without much difficulty.

1. Introduction

Biological cells may go through the process of lysis in which the cell's membrane ruptures and the cytosol dilutes in the surrounding medium. The degree of ease with which cells are lysed can be a measure of their integrity and health [1,2]. The endurance of blood cells, such as erythrocytes or lymphocytes, to some specific stresses, may give valuable information about the cells that may help diagnose some health disorders. For instance, determining the resistance of erythrocytes to an osmotic stress, which may cause lysis, depending on the state of cells, can be used in the diagnostics of certain common diseases [2–4]. Therefore, having sensors to monitor the process of cell lysis under a controlled stress could be used in developing new medical diagnostics tools.

Currently, there are many techniques to measure the number of cells lysed during processes such as the addition of solvents or enzymes that disrupt the cells' membranes, or through mechanical perturbations by using a blender or sonication [5–8]. These techniques can be divided mainly in optical and electrical. The optical techniques are the most popular in the medical area, but they are generally time-consuming [4,9]. On the other hand, electrical methods are arising as an alternative. Recent works show significant advances in electrical measurements combined with microfluidics technology applications [10–16]. These works have focused mainly on single-cell measurements at a fast rate so that a large number of cells can be analyzed in a reasonable time. Although single-cell studies can give information regarding what is occurring locally inside a cell, for developing medical diagnostics tools it should be more convenient to monitor a large number of cells and obtaining average properties in a simple and rapid way. Also, the average electrical response of a volume of suspended cells can be readily interpreted to infer average properties of cells with well-established theories.

Monitoring in time a cell-lysis process *via* impedance measurements has already been addressed in Refs. [12, 13]. In these works, impedance spectroscopy measurements were obtained a few times during a lysis process in a cell suspension demonstrating the viability of using electrical impedance measurements to distinguish different stages of the lysis process. However, in order to obtain more detailed information about the kinetics of a lysis process it would be needed to monitor the process continuously in time. Although time-continuous spectroscopy or multi-frequency impedance measurements are viable, the cost and complexity of such a device would be considerable. For continuous time monitoring, single-frequency impedance measurements are more appealing and should be the first option to be considered.

The electrical properties of cell suspensions and tissues have been studied for many years. Reviews of the electrical properties of tissues

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https://doi.org/10.1016/j.sbsr.2018.09.001
Received 30 May 2018; Received in revised form 3 September 2018; Accepted 6 September 2018

and cell suspensions, overviewing several polarization models of biological cells, and their frequency dependence, have been written in the past. See for instance Refs. [17-22]. Different mechanisms of cell's electrical polarization have been identified and studied. Generally, three dispersion bands are recognized. Namely the α, the β and γ dispersion bands [18,21–23].

The cell's polarization within the α dispersion band at the lower end of radiofrequencies, arise from the perturbation of free charge carriers piled up around the membrane of cells. The mechanisms for this type of cell polarization are more complicated and less well-understood [17,23–26] than those generally responsible for the β and γ dispersion bands. Modeling the α dispersion band require more sophisticated theoretical approaches. Within the β dispersion band, the main mechanism of cell's polarization is the so-called Maxwell-Wagner (M-W) effect which is dependent of the cell's membrane and morphology, and the conductivity contrast between the interior fluid of the cell (cytosol) and the exterior medium. The γ dispersion band is due to the relaxation of the polarization of water molecules and may be some other biological molecules, but these are less dependent on the cell's condition. The frequency range where the α, β and γ dispersion bands occur will in general depend on the type of cells and the medium in which they are suspended.

Several electrical sensors have been studied for their use in determining the volume fraction of cells in suspensions, see for instance [27–38]. However, the conclusions in those references do not help understand how to sense electrically the lysis of biological cells. Previous works focused on how sensitive is the impedance of an electrical sensor filled with a cell suspension to the addition or removal of intact cells. However, as it will become clear in this paper such measurements are not equivalent to measuring the cell's volume fraction decrease during a cell-lysis process. For instance, upon adding or removing cells in suspension the total number of ions in the suspension changes, whereas during a lysis process the number of ions in solutions remains constant. Thus, it is not evident whether the resistivity of the suspension should change over time during a lysis process.

In this paper we aim to assess theoretically whether a simple electrical sensor measuring continuously in time at a single frequency can have enough sensitivity to monitor the kinetics of a cell lysis process and, more specifically, whether either the capacitance or resistance provide an appropriate signal to sense the progress of cell lysis. Although in the α band the electrical response to cell lysis may be expected to be quite strong, we focus our attention only for frequencies in the range of the so-called β dispersion band. The reason is that, as already mentioned, the electrical polarization phenomena within the α band is appreciably more complex and depend on more physical parameters than in the β dispersion. Therefore, we do not consider frequencies within the α band suitable for sensing a lysis process. For concreteness in our modeling we use some specific values of the type and density of ions, cell's membrane properties, and we consider only spherical cells. Despite these specific assumptions we are able to reach some general conclusions for single-frequency impedance sensing for monitoring cell lysis. Of course, in particular cases more involved modeling may be necessary depending on the specifics of a cell suspension. Nevertheless, finding out whether one can sense electrically cell lysis and the order of magnitude of the resolution that can be expected, is necessary for further cell-lysis sensor research. Ultimately, we aim to provide an alternative and more robust class of sensors that may be used in medical diagnostics applications.

This paper is organized as follows. In Section 2 we provide the theoretical formulae used to estimate the relative sensitivity of the capacitance and resistance of an electrical sensor during a lysis process in a cell suspension. In Section 3 we provide representative values for the sensitivity of the capacitance and resistance during a cell-lysis process. A comparison with the sensitivity of an electrical sensor to simply adding or removing cells is discussed. In Section 4 we estimate the resolution in the volume of lysed cells achievable in practice with standard instrumentation, and finally, in Section 5 we present a discussion of the results and our conclusions.

2. Theoretical formulae

Inside and outside biological cells there are ionic species in solution. These are free charge carriers that may displace when an electric field is applied. When charges carriers reach an interface that they cannot cross, there is charge accumulation at the interface. Free charge accumulation at the interface between electrolyte solutions is referred to as the M-W effect. In the case of biological cells immersed in an oscillating electric field, the free charge accumulation at opposite sides on the surface of the cell's membrane and around the cell, give rise to a net induced oscillating electric-dipole, which in general will be out of phase with respect to the applied electric field. The induced dipole contributes to the effective dielectric function of the whole medium in much the same way as the induced dipoles on the molecules of a homogeneous medium contributes to the electrical permittivity.

The electrical response of a cell's suspension can be summarized in its effective electric permittivity, $\epsilon_{eff} = \epsilon_{eff}' - i\epsilon_{eff}''$. Here we will denote the imaginary unit as i, and the real and imaginary parts of a complex function with a single and a double quotation mark, respectively. In many texts dealing with low frequency electrical properties of materials, the conductivity is treated independently of the electric permittivity, however, in this paper, as in many others, the conductivity is included in the imaginary part ofϵ_{eff}. In general, the definition of the electric permittivity of a material is a matter of convention. Commonly one refers to the dielectric function of a material. This is a dimensionless quantity and is given by the electric permittivity of the material divided by the vacuum's electric permittivity, ϵ_0. We will denote dielectric functions by adding a tilde on top of the quantity. Thus, the effective dielectric function will be denoted as, $\tilde{\epsilon}_{eff} = \tilde{\epsilon}'_{eff} - i\tilde{\epsilon}''_{eff} \equiv \epsilon_{eff}/\epsilon_0$. The effective dielectric function of a material can be readily deduced from impedance measurements of parallel-plate capacitor devices hosting the biomaterial. In the following subsections we relate measurable electrical parameters with the effective dielectric function of a cell suspension.

2.1. Electrical parameters and the effective dielectric function

Then, let us consider a parallel-plate capacitor of electrodes' area A and separated a distance d. Let us suppose the capacitor is filled with a cell suspension, that the cells are randomly positioned in the space between the electrodes and that the number of cells is large enough that we can define properly an effective electric permittivity for the cell suspension as indicated in Fig. 1. An equivalent circuit consists of a capacitor C with a parallel resistor R as illustrated in Fig. 1.

The frequency-dependent capacitance and parallel resistance of the equivalent circuit are given by,

$$C(\omega) = \frac{\epsilon_0 \tilde{\epsilon}'_{eff}(\omega) A}{d}, \tag{1}$$

$$R(\omega) = \frac{d}{\omega \epsilon_0 \tilde{\epsilon}''_{eff}(\omega) A}. \tag{2}$$

The latter formulas are for an ideal parallel-plate capacitor but using an effective value for the area A takes into account fringing field effects at and near the borders of the capacitor [39–41]. Typically, A is determined from experimental measurements. We should note that in practice, effects of electrode polarization may be important when measuring C and R at lower radiofrequencies. In this case a capacitor must be added in series to the equivalent circuit of the sensor shown in Fig. 1. There are several techniques to compensate for electrode polarization, however, these are out of the scope of this paper. For a