Sensitivity of Dielectric Spectroscopy of Erythrocyte Suspensions with different glucose concentrations

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Dielectric spectroscopy is assumed to offer the potential of non-invasive detection of changes in cell size and shape or the monitoring of physiological processes in cell suspensions. On a larger scale there are different approaches to apply it to transcutaneous monitoring of physiological changes for medical diagnosis. Changes in the dielectric response of erythrocytes in solution to different external glucose concentrations have been previously reported in literature. In this study investigated this potential effects are investigated by two different experimental systems both based on impedance spectroscopy. The first system features a differential measurement of cell suspension above interdigitated microelectrodes. The second system is based on flow cytometry measurement consisting of a microfluidic chip including electrodes at the top and bottom of its microfluidic channel, allowing for single cell characterization. Both measurement systems use an impedance analyser in the frequency range from 10 kHz up to 100 MHz in discrete steps. The measured sample consists of biconcave erythrocytes from rats soluted in Phosphate buffered saline (PBS). The PBS containing different concentration of glucose is balanced with Mannitol to avoid impedance and osmotic pressure changes. Preliminary measurement results suggest no significant direct correlation between the glucose concentration and the impedance spectra of erythrocytes within the given measurement sensitivity.

1. INTRODUCTION

Dielectric spectroscopy offers the potential of non-invasive detection of changes in cell size and shape or the monitoring of physiological processes in cell suspensions. On a larger scale there are different approaches to apply it to transcutaneous monitoring of physiological changes for medical diagnosis \cite{1}. In both cases, the dielectric properties of a highly non-homogeneous medium are measured by specific sensor geometries averaging over a specific measurement volume. Knowing the sensor geometry and the dielectric properties of the target medium, the shapes of cells or the dielectric properties of cell constitutes such as the membrane or the cytoplasm can be determined.

Recent studies outlined the hypothesis that the D-glucose concentration in blood plasma affects the dielectric properties of erythrocytes \cite{2,3}. In fact some studies concluded from dielectric spectroscopy measurements that the membrane capacity of the erythrocytes is related to the external glucose concentration. Furthermore it was demonstrated by \cite{4} that the electric parameters of cytoplasm are not affected by external glucose. This study investigated this hypothesis by two different experimental systems both based on impedance spectroscopy. The measurements uncertainties are evaluated and detection limits are computed with the help of numerical simulations.

Figure 1. CAD sketches of the two experimental setups together with light microscopy pictures of erythrocytes used for measurements
2. MATERIALS AND METHODS

Two different experimental systems are used for the measurements (Figure 1) both based on the principle of differential dielectric spectroscopy, i.e. the difference in impedance of a cell electrolyte solution to the pure electrolyte solution. The first system features cells in suspension above 10 planar interdigitated microelectrodes with an electrode to gap width of 175 to 50 um. The second system design by Leister Process Technologies, Axetris Division (Kaegiswil, Switzerland) consists of a microfluidic chip including electrodes at the top and bottom of its microfluidic (20 x 20 um) channel [5]. The first system is used to measure changes in cell suspensions whereas the second on allows for single cell characterization. Both measurements are conducted by an impedance analyser in discrete frequency steps at 0.5 and 9.0 MHz. Blood sample from a healthy rat withdrawn by heart puncture was collected Erythrocytes were isolated by centrifugation for 2 minutes at 460 rcf and supernatant elimination. Phosphate saline buffer (PBS), D-glucose and Mannitol isotonic solutions were prepared and mixed at different D-glucose concentrations (0mM, 5mM, 10mM, 20mM) while keeping osmolarity and conductivity constant. The osmolarity was measured individually for each solution at 291 ± 1 mOsm. The cell suspension experiments were conducted 5 times whereas the flow cell analysis was performed 6 times. The resulting impedance differences \( \Delta z_{XM, j} \) were normalised to the mean complex impedance difference \( \Delta z_{0M} \) for 0 mM glucose concentration using the following formula: \( \Delta z_{XM, j} = \left( \Delta z_{XM, j} - \Delta z_{0M} \right) / \Delta z_{0M} \). The resistance / reactance deviations denote its real / imaginary part, respectively. Each group was tested for normal distribution using the Chi-square test and statistical analysis was performed with ANOVA method.

The flow cell configuration is numerically analysed (Fig. 2) using the linear 3D quasi-electrostatic finite element solver of the commercial simulation platform COMSOL (Version 4.2). The flow cell in conjunction with a simplified geometrical representation of an erythrocyte based on elliptical functions is modelled as a two-phase system (external media and cytoplasma) in combination with an impedance boundary layer to emulate the cell membrane. The complex and dispersive dielectric material properties of the cell constitutes are summarised in [7]. However, the uncertainty of the dielectric parameters used is large due to the complexity of measuring individual cell parameters. Furthermore, the considered simplified model does not account for nonlinear effects, like chemical processes in the membrane, which can significantly alter the measurement signals. Moreover, it is assumed that the cell shape is not affected by the applied electric field. The validity and the uncertainties of the numerical model are discussed in [6].

3. PRELIMINARY RESULTS

Despite all uncertainties regarding the dielectric parameters of the simulation model the numerical results depicted in Fig. 2 show first of all that the detection limits for a change in the membrane capacity is roughly 50 fold smaller than a comparable change in external media conductivity. Even a similar change in cytoplasmic conductivity requires a 10 times higher sensitivity. Overall is the measurement standard deviation outlined in Fig. 3 / 4 due to variation in cell size, shape and position is around ±10% for the cell suspension analysis and around ±20% for the flow cell analysis, thus requiring a high number of experiments for statistical significant statements. For example in order to detect an reactance deviation of 1% with an measured standard deviation of 20% with an significance of 99% more than 21 independent experiments are necessary. Furthermore the measurement results for 500 kHz (Fig. 3) with an major change in the resistance deviation indicate rather an change in external media conductivity than in membrane capacity or an superimposition of both.
4. CONCLUSION

Preliminary measurement results suggest no direct correlation between the glucose concentration and the impedance spectra of erythrocytes within the measurement sensitivity. Furthermore, any change in the dielectric response of single cells caused by alteration of the membrane capacity around ±25% requires a high number of independent experiments due to variance in shape, size, and position of the cell. But these preliminary results are based on a rough estimation and more elaborated simulation analysis of individual cell features have to be conducted. The results of the numerical simulations can be used to determine the number of repetitions needed given a measured standard deviation in order to achieve significance.

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REFERENCES