NANOSCALE IMPEDANCE MICROSCOPY ON SINGLE BACTERIA. A THEORETICAL STUDY.

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In recent years it has been recognized that single cell studies with microbial cells, as compared to the more common population based studies, may provide answers to some unresolved scientific questions [1]. Most of the advances reported until now have been produced with microorganisms with relatively large sizes (yeast cells, algae, amebae, etc.) of at least 5 μm in diameter and hence accessible by optical techniques and conventional micromanipulation technologies at the single cell level [2]. Much less has been done with small bacteria with typical sizes around 1 μm which lye at the frontier of conventional techniques and hence require more advanced (nano)techniques, essentially Atomic Force Microscopy (AFM). [3,4,5] This technique has allowed obtaining three dimensional images of the live bacterial cell surfaces with high spatial resolution as well as quantification of adhesion to molecules and surfaces, the study of the antibacterial effect of different compounds, evidence for horizontal genetic transfer through conjugative pili, DNA-protein interactions, etc. In spite of these results, still a lot remains to be explored in order to better understand the properties of these small single bacterial cells.

In our groups we are exploring novel applications of atomic force microscopy to the study of single bacterial cells to generate new biologically relevant knowledge not currently available by existing biotechniques. In particular, we aim to combine AFM and nanoscale impedance electrical measurements to rapidly discriminate between the most relevant and prevalent pathotypes of some pathogens. We present in this contribution a theoretical study to assess the experimental viability of this approach. The theoretical study has been performed at both the whole cell level and at the local cell level. In the former case (Fig. 1a) analytical parallel plate models have been used, while in the later case (Fig. 1b) finite element numerical simulations including the system geometry (AFM probe and bacteria) have been used. The bacteria have been simply modelled as a closed sphere of 500 nm in diameter surrounded by an insulating membrane 6 nm thick with intrinsic electric properties represented by the membrane dielectric constant, cytoplasm conductivity, and cytoplasm dielectric constant. The computed magnitude (in analogy to the experiments to be performed) consists of the difference in impedance corresponding to the electrode-bacteria and electrode-air system. The impedance differences as a function of frequency and for a range of physiologically relevant set of values of the intrinsic electric properties have been computed and compared to the specifications of our state of the art wide bandwidth high gain current sensor for impedance measurements [6].

The main conclusion of the study is that in the frequency range accessible to our measuring set up (100 Hz-1 MHz) and under the dry conditions considered, nanoscale impedance measurements are mostly sensitive to the dielectric properties of the bacterial membrane, and very little to the cytoplasm properties. This fact is illustrated in Fig. 2a where the capacitance difference is plotted as a function of the frequency for various membrane dielectric constants and in Fig. 2b (top) where the values at 100 KHz are plotted as a function of the dielectric constant of the membrane. Due to the high sensitivity of our measuring set up, relative variations as small as ~10-15% in the membrane relative dielectric constant might be detectable Fig. 2b (bottom), thus being sensitive enough for phenotype variation associated to membrane changes.

References:

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Figures:

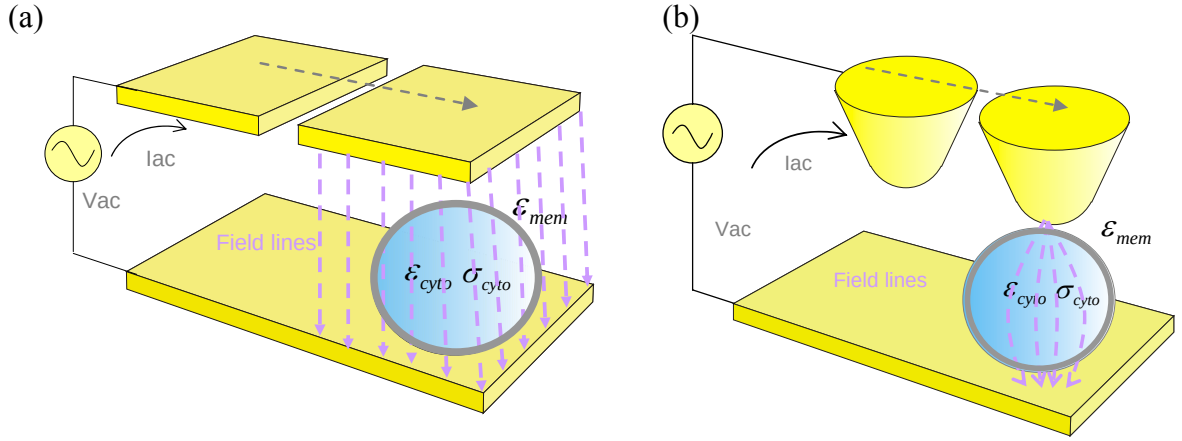

Figure 1: a) Schematic representation of the systems modelled. (a) Whole cell approach (parallel plate configuration), where analytical models can be used and (b) local cell approach (tip-substrate configuration) where finite element numerical simulations are used. The bacteria are simply modelled as a sphere of diameter 500 nm with a 6 nm membrane. The intrinsic electric properties of the membrane are represented by the membrane dielectric constant, cytoplasm conductivity and cytoplasm dielectric constant.

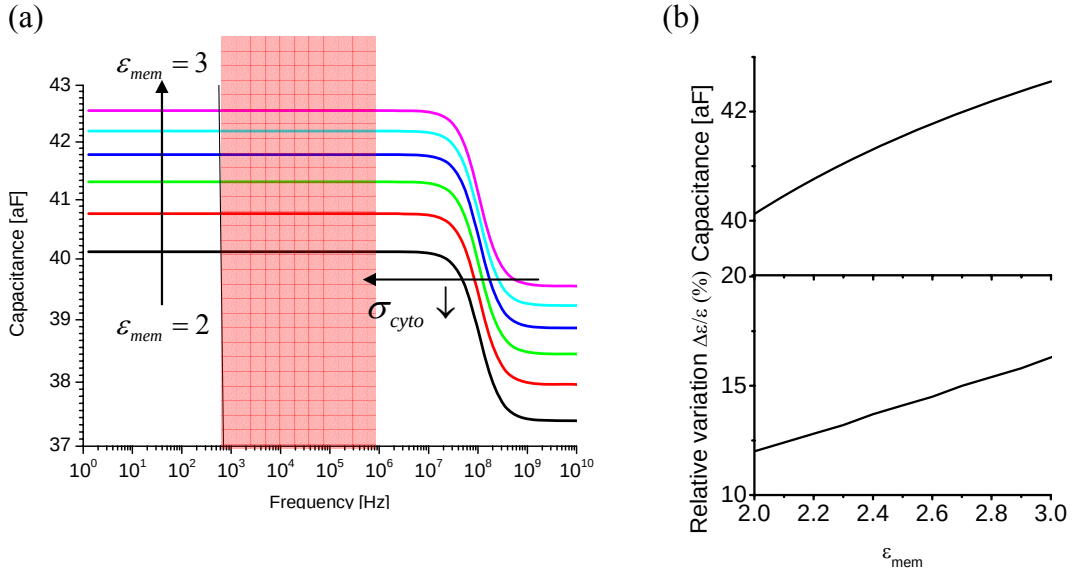

Figure 2: Change in the capacitance (imaginary part of the admittance divided by the frequency) as a function of frequency for different values of the dielectric constant of the membrane. The effect of reducing the cytoplasm conductivity is indicated schematically by the horizontal arrow. The shadow area corresponds to the measurable region. (b) Capacitance plateaux values in the range from 10 kHz to 1 MHz vs relative dielectric constant of the membrane for a cytoplasm conductivity of 0.1 [S/m] and cytoplasm permittivity of 80. (c) Minimum detectable variation in membrane dielectric constant (expressed in relative variation) for different values of the dielectric constant of the membrane (assumed experimental conditions: applied voltage 1 V_{rms}, measuring time per point 1 s, frequency range from 10 kHz to 1 MHz, detector capacitance noise under these conditions 0.83 aF).