Electric polarization properties of single bacteria measured with electrostatic force microscopy

Theoretical and practical studies of Dielectric constant of single bacteria and smaller elements

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DOCTORAL THESIS

1 Introduction

The present thesis is included in the broad field of Nanotechnology. This term refers to all kind of technology (and science) that addresses the nanometer scale range (usually < 200 nm). As such, this term is somewhat vague as it encompasses many scientific disciplines, such as, physics, engineering, chemistry, pharmacology, biology, medicine and a large etcetera. In particular this thesis has been carried out in the narrower field of Nanobiotechnology, in which the systems under study belongs to the Biology realm, ranging from biomolecules and viruses to bacteria and eukaryotic cells. And since most of my research has been carried out about electric properties, we can even add a narrower term: Nanobioelectricity to define the field of research in which this thesis is centered.

As most Nanotechnology fields, Nanobioelectricity is a multidisciplinary field with contributions from physics, biology, electrical engineering and, even, mathematics. One of the biggest limitations in many Bio-physical interdisciplinarity research is that too often happens that the research manly consists in biologists providing to physicists or technologists with simple biological test samples for proof-of-concept experiments.

Interdisciplinary research requires of the involvement of various research groups belonging to different scientific areas with the aim of combining both expertises in order to solve scientific problems that cannot be resolved by using the knowledge or techniques belonging to the groups separately. This definition of interdisciplinarity should imply the use of advanced techniques from all the groups, which combined together, provide an added value to the collaboration.

This is particularly true in the case of nanotechnologies applied to biology, in which it is really surprising to notice the small amount of new biologically relevant knowledge generated by applying nanotechnologies to biology, as compared to the potentialities redundantly mentioned in the scientific papers.

One of the scopes of this thesis was then to try to overcome some of the limitations of previously existing works in the Nanobiotechnology field with the objective to achieve novel biologically relevant information by the use of advanced nanotechnolgies. For this purpose, we focused our efforts in the development of novel nanotechnologies to address the electrical properties of single bacteria cells, in view of the lack of techniques existing for this purpose.

The research in single bacteria cells, as compared to colony studies with millions of bacteria, can provide novel and important insights into the bacteria behavior. For instance, individual cells within clonal microbial cultures exhibit remarkable phenotypic heterogeneity, i.e. in spite of having the same genetic content their response to the same environment provides different observable characteristics related to morphology, development, biochemical and physiological properties, or behavior. The heterogeneity at the single-cell level is typically masked in conventional studies of microbial populations, which rely on data averaged across thousands or millions of cells in a sample.

In recent years it has been recognized that single cell studies in microbiology may provide answers to some unresolved scientific questions [1]. By looking in detail to the advances achieved so far and the techniques used to achieve them [2], one realizes that most of the advances have been produced in microorganisms with relatively large sizes (yeast cells, algae, amebae, etc.) of around 5 μm in diameter and hence accessible by optical techniques and conventional micromanipulation technologies at the single cell level.

Much less has been done with single bacteria with typical sizes around 1 μm which lie at the frontier of conventional techniques and hence require more advanced (nano)techniques. In this case, most of the investigations have been carried out by means of Atomic Force Microscopy (AFM) [3], [4], [5]. This technique has allowed to obtain three-dimensional images of the living 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, several points remain to be explored in order to help biologists to

understand better the properties of single bacterial cells. In particular, practically nothing is known about bacterial cell electrical properties measured at the single cell levels. The electrical properties of bacterial cells can inform on properties of the cells which cannot be achieved by other techniques (e.g. mechanical or optical techniques). For instance, some results have suggested that small changes in the structure of bacteria (e.g. the expression or not of a given protein) can lead to changes in the response of bacteria to alternating electric fields (dielectrophoresis) [6].

The objective of our work is to implement an electrical AFM based methodology able to map at the nanoscale the intrinsic electric properties of single bacterial cells (e.g dielectric constant), thus allowing us to resolve subcellular features in a label-free way.

Being able to measure the electric polarizability – which indicates how it reacts to an external electric field – of a single bacterial cell can shed light on the biochemical constituents of the bacterium, as well as on their internal structure, thus opening new possibilities for analytical studies and new explorations to evaluate their critical biological properties, such as adhesion, virulence or viability.

To reach this purpose I had gone through the basics of microbiology (to know their biochemical composition, structure, morphology and metabolism) to advanced Scanning Probe Microscopy techniques.

Starting from my initial background in Atomic Force Microscopy (AFM) (mainly for topographic imaging and some electrical measurements, basically conductive AFM), I specialized in Electrostatic Force Microscopy (EFM), the technique that was finally chosen since it was more suitable for the desired purposes. In particular, I contributed to the work of the research group, mostly on the modeling part, to set up a new methodology (theoretical and experimental) based on EFM that enabled us to obtain the intrinsic dielectric permittivity of three-dimensional objects (from nanometers to microns). The methodology had been hitherto mainly used in planar films (thin and thick). With the developed technology, and after its validation with inorganic samples bearing some similarity to bacteria cells, I devoted most of my efforts to adapt it to the study of single bacteria cells. During the thesis, I applied

the developed methodology to four different bacterial types - *Lactobacilus sakei*, *Salmonella* Typhimurium, *Escherchia coli* and *Listeria innocua* - all of which are of either clinical or industrial relevance. We wanted to see whether their permittivities were different and how they were related. By chance we observed a large dependence of this permittivity depending on the hydration of the cells. So an explanation was proposed relating the gram negative and gram positive bacteria with the hydration state. The hypothesis was that the cell wall and cell membranes could have a large influence and new core-shell models were proposed.

In addition to the bacteria work, some prospective work was also carried out on other smaller biological objects (like viruses and nanoparticles), for which I developed some finite element modeling methodologies to substitute previous analysis based on analytical formulas.

The structure of the thesis is organized in *twelve* chapters as follows. After the introduction, in the *second* chapter we make a general introduction to the structure and composition of the bacterial cell. In the *third* chapter we present the basics of Atomic Force Microscopy and of the electric measurements based on it, making a special emphasis in Electrostatic force microscopy. The *fourth* chapter is dedicated to the principles of quantitative Electrostatic Force Microscopy and its use in the extraction of the dielectric properties at the nanoscale (using known permittivity samples for validation). In the *fifth* chapter we present the experimental methodology used for the study of the dielectric properties of single bacteria cells, while in the *sixth* chapter the theoretical approach is developed. In the *seventh* chapter we present the application of these methodologies to a systematic analysis of the dielectric properties of single bacteria cells. In *eighth* chapter a prospective modeling analysis of the applications of these techniques to smaller scale objects like viruses and nanoparticles is made. Finally in the *ninth* chapter we will conclude and give a short overview on future perspectives. Chapters *tenth*, *eleventh* and *twelfth* are dedicated for appendix, references and summary in Catalan, respectively.