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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9 Conclusion

Previous electrical studies – which until now have only been able to be done on bacterial populations involving millions of bacterial cells, and not on single cells – have allowed researchers to detect bacteria in an environment, count and differentiate them, determine their viability, distinguish mutants even among highly similar genotypes, and separate them from other cells. A novel technique based on electrostatic force microscopy has been presented in this thesis, which allows addressing the electrical properties of bacterial cells at the single cell level. The fact that this new technique allows the measurement of the electrical properties of single cells without the need for separation would enable in the future the heterogeneity within a population to be accurately quantified.

I used quantitative electrostatic force microscopy (EFM) in the study, a technique that our group has recently developed and successfully applied to measure the electrical properties of 3D nano-objects such as nanoparticles and viruses. EFM senses the electrical properties of the whole bacterial cell, including its cytoplasmatic region, and so is able to provide a wealth of information on a bacterium's electrical response.

To that end a new methodology had to be applied to work with bacteria which are in another topographical range (around the μm). One of the main challenges overcome during this thesis was in the theoretical modeling of the tip bacteria interaction, an intrinsically 3D large scale problem. After a systematic analysis of many different configurations, including some analytically workable, we reached the conclusion that the tip bacteria system could be accurately modeled using a quite simple 2D model consisting of cone truncated to a tangent sphere over an oblate hemi-spheroid. This simplified theoretical approach was key to achieve many of the results of this work of thesis.

Using both the experimental and the theoretical methodologies we obtained the results of a known dielectric sample with height similar to bacteria (200nm) and large lateral size (to avoid finite size effects). The nominal value of this material, Si_3N_4, is ε_r = 6-8 and the obtained value was ε_r = 7.65 thus validating the methodology. This study also served to demonstrate that two measuring methodologies used so far in the group, namely the constant height and the force-distance approaches, gavethe same results and either one could be used to our convenience.

In this way, we later quantified the electric polarization response of four bacterial types - *Lactobacilus sakei*, *Salmonella* Typhimurium, *Escherchia coli* and *Listeria innocua*, all of which are of either clinical or industrial relevance – and revealed important differences between Gram-negative (G-) and Gram-positive (G+) bacteria. We obtained ε_r = 3-5 (for both Gram types) when analyzed in dry conditions and ε_r = 6-7 (G-) and ε_r = 15-20 (G+) when analyzed in a humid environment and we proposed a two-shell model that could explain this situation.

Comparing bacteria with smaller objects (nanoparticles, viruses, etc.) we find that for the latter a phenomenological equation could be derived, although in the ranges it was set it is not possible to be applied to the bacteria case.

Something similar happened with the two shell model, where small objects can be approximated by an analytical formula because the electrical field generated by the probe is approximately homogeneous in the part that mostly contributes to the force. It is something that does not happen in larger objects like bacteria (in the μm range).

Finally we concluded that for the smallest particles analyzed (i.e. viruses or nanoparticles) the eccentricity plays a very important role. The topography has to be precisely measured. In contrast it is not as important when studying bacterial cells, as long as measurements are performed at its centre. That is the reason why an axial symmetry of a cone truncated to a tangent sphere over an oblate hemi-spheroid could be used.

All together the results obtained in this work of thesis demonstrates the enormous potential of electrical Scanning Probe Microscopy techniques applied to the study of single bacteria cells.