DIELECTROPHORETIC MICROSYSTEM FOR SEPARATION OF DOUBLE MUTANT BACTERIA

M. Castellarnau*, N. Zine**, J. Bausells**, C. Madrid*, A. Juárez*, J. Samitier*, A. Errachid*

* Nanobioengineering Research Laboratory (CREBEC), Barcelona Science Park, Barcelona, Spain.
** Centro Nacional de Microelectronica (IMB-CSIC), Campus UAB, Bellaterra, Spain.

mcastellarnau@pcb.ub.es

Abstract: A dielectrophoretic microdevice has been used to characterize the dielectrical properties of two kinds of bacteria suspensions. *Escherichia coli* with the double mutation (hha-hns) can be distinguished from reference suspensions of *E.coli* strain 5K. Moreover, bacteria separation in a mixed suspension has been achieved by dielectrophoresis.

Introduction

Infectious diseases are human illnesses caused by viruses, bacteria, parasites, fungi and other microbes. They may be spread by direct contact with an infected person or animal, by ingesting contaminated food or water, by insects, or by contact with contaminated surroundings like animal droppings or even contaminated air. Societal costs of infectious diseases are staggering. In the United States, the yearly price tags of bacteria infectious diseases are $30 billion for intestinal infections and $1 billion for salmonella [1].

Conventional bacteria inspection methods, such as the colony counting technique, are well established and reliable. However, they cannot provide a fast diagnosis in case of emergency because they require rather long times for bacteria incubation (typically a few days).

A reliable, faster alternative can be based on AC-electrokinetic techniques, such as dielectrophresis (DEP) and electrorotation (ROT), which have widely been used for handling, separation and characterization of particles and biological samples (such as latex beads, yeasts, bacteria or DNA) [2-5].

When a dielectric particle is placed under the influence of an AC field, a dipole moment is induced within the particle [6,7]. In the case of a non uniform field the polarized particle experiences a translational force, known as a dielectrophoretic force. This force depends on the relationship between the dielectric properties of the particle and the surrounding medium, and on the magnitude and frequency of the applied electric field. For spherical particles the time-averaged DEP force is [8]:

$$\mathbf{F}_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m \,\mathrm{Re}\left[K(\omega)\right] \nabla \left|E_{rms}\right|^2 \qquad (1)$$

where r is the particle radius, ε_0 is the permittivity of free space, ε_m is the real part of the permittivity of the suspending medium and E_{rms} is the r.m.s. electric field. The factor $K(\omega)$ (the Clausius-Mossotti factor) depends on the complex permittivites of both the particle and the medium and is a measure of the effective polarizability of the particle. In the case of spherical particles this factor is given by:

$$K(\omega) = \frac{\left(\varepsilon_p^* - \varepsilon_m^*\right)}{\left(\varepsilon_p^* + 2\varepsilon_m^*\right)}, \quad \underset{(i=p,m)}{\varepsilon_i^*} = \varepsilon_i - j\frac{\sigma_i}{\varepsilon_0 \omega} \qquad (2)$$

where the indices p and m refer to the particle and the medium, respectively. ε and σ are, respectively, the permittivity and the conductivity of the dielectric, ω is the angular frequency of the applied filed $(\omega = 2\pi f)$ and $j = \sqrt{-1}$. From this, two situations can be defined depending on the sign of the real part of the Clausius-Mossotti factor: when Re[$K(\omega)$]>0 the particles move to a region of a maximum electric field, defining a positive dielectrophoresis situation (p-DEP). Conversely, when Re[$K(\omega)$]<0, a negative dielectrophoresis situation (n-DEP) is produced and particles are repelled from these regions. Typically, for spherical particles, Re[$K(\omega)$] has a value in the range from -0.5 to 1.

Conventional dielectrophoresis (c-DEP) measurements consist of determine the crossover frequencies (frequencies at which the dielectrophoresis force is null), at different values of medium conductivity. Studying the evolution of the crossover frequencies with the medium conductivity, it is possible to compare and distinguish cells with different dielectric properties.

In this work, we present some results concerning the characterisation and separation of bacteria suspensions by conventional dielectrophoresis. In particular, we are able to distinguish between genetically modified bacteria (*Escherichia coli* hha-hns with and without conjugative plasmid R27, which makes the bacteria antibiotic resistant) and non-modified bacteria (*Escherichia coli* strain 5K).

Materials and Methods

Dielectrophoretic forces can be obtained using a microdevice which consists of a pair of electrodes in a U- and T-shape that allows the generation of an inhomogeneous electric field [9]. The terminals of these electrodes are connected to a signal generator (Agilent 33250A for low frequencies or HP8657A for high frequencies) which generates AC signals of 6 - 10 V

ISSN: 1727-1983

peak-to-peak with a variable frequency in the 10 kHz - 200 MHz range.

Bacteria suspensions of *Escherichia coli* in distilled water have been used as biological samples for the dielectrophoresis experiments. Comparison has been made between *Escherichia coli* strain 5K [10] and the double hha-hns mutant with and without plasmid R27 (strain 5K hha-hns). *Escherichia coli* cells were grown at 37ºC in LB agar medium for 24 h. The double mutants with plasmid R27 are also grown at 37ºC in LB agar medium with added tetracycline 15 µg/m for 24 h, they are then centrifugated and resuspended in distilled water to obtain the desired concentration. Sample conductivity was controlled by progressively adding aliquots of NaCl to the sample solution, and measured with a conductivity-meter (Corning conductivity meter 441). Finally, particle movement and manipulation were observed using a Nikon Eclipse L150 reflectance microscope fitted with a digital camera. Images were recorded using a personal computer via an image acquisition card.

A multi-shell model has been used in order to describe the dielectric behaviour of the bacteria suspensions, as shown in Figure 1. A 2-shell ellipsoidal model with a cylindrical section, which consists on an inner cytoplasm surrounded by a membrane and a cell wall, has been adapted from Führ's model parameters obtained by electrorotation [11]. The model considers the dielectric parameters (complex permittivity) of the surrounding medium and the different layers that make up the cell, as well as the surface conductance of the cell due to the double layer at the interface with the medium [12,13].

Considering the bacteria as spheroid with a cylindrical cross-section implies some variations in the Clausius-Mossoti factor expression, producing new values for Re[$K(\omega)$] which can be outside the -0.5 to 1 range.

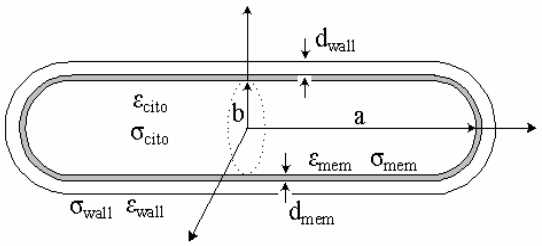

Figure 1: Diagram of the ellipsoid 2-shell model considered for the bacteria.

A sensitivity study of model parameters has been completed in order to determine the influence of these parameters on the evolution of the real part of the Clausius-Mossotti factor with the frequency of the applied field. Studying a medium conductivity range from 1µS·cm^{-1} to 1200µS·cm^{-1}, two frequency regions can be defined, as shown in Figure 2. The first region, below 10 MHz, is governed by the dielectric parameters of the cell membrane, and at a certain medium conductivities, a first crossover frequency is observed (fc1). At higher frequencies, above 10 MHz, a second region is defined which mainly depends on the dielectric parameters of the cytoplasm but also includes that of the cell membrane, and a second crossover frequency is observed (fc2).

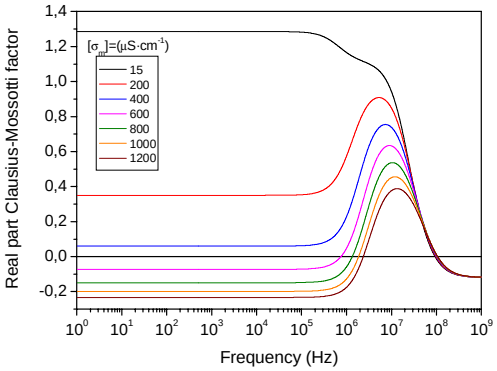

Figure 2: Evolution of the real part of the Clausius-Mossoti factor with the frequency of the applied field, for different medium conductivities, considering 2-shell model for bacteria.

Results

A comparison of the evolution of the crossover frequencies with the medium conductivity has been made using the 3 bacteria suspensions. For the medium conductivity in the range 1µS·cm^{-1} to 1200µS·cm^{-1}, two frequency regions can be defined. As the model predicts a first crossover frequencies (fc1) are observed, below 10MHz, for medium conductivities higher than 500µS·cm^{-1}. The second crossover frequencies (fc2) are observed in the high frequency range, 10 MHz - 200 MHz, for all the medium conductivities studied.

The experimental results have been compared with data calculated using a 2-layer electrical model for the bacteria [11]. Figure 3 shows that differences exist between the crossover frequencies, at a given medium conductivity, when comparing the 5K strain with the *E.coli* double mutant bacteria, with and without the conjugative plasmid R27.

At high frequencies, bacteria separation by c-DEP has been achieved using a suspension with a 50/50 mixed population of *E.coli* strain 5K and of hha-hns double mutants, (Figure 4). Initially, both kinds of bacteria were trapped at the electrode edges under a p-DEP situation by applying an AC signal of 8Vpeak-to-peak at 10MHz. Once the bacteria were locally trapped, the frequency was switched to 80MHz and a local separation of the *E.coli* strain 5K and the double mutant bacteria occurs. When the stationary stage is reached, the *E.coli* strain 5K remained trapped at the electrodes by p-DEP while the hha-hns double mutant bacteria were repelled by n-DEP.

ISSN: 1727-1983

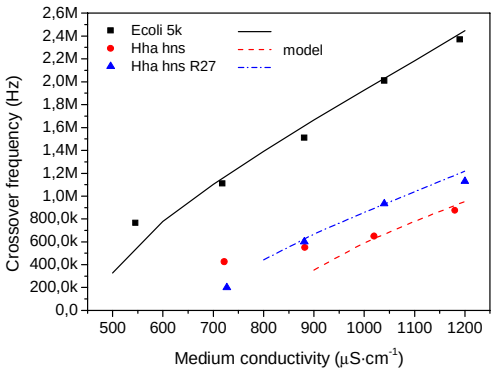

Figure 3: Evolution of the crossover frequency with the medium conductivity, for the 3 bacteria suspensions: (top) in the low frequency region; (bottom) in the high frequency region.

Discussion

The 2 shell model descried above has been adjusted to the data obtained from c-DEP measurements. From this adjustment it is possible to qualitatively describe the relative variations in the dielectric parameters of the model to compare the results for the different kinds of bacteria. The geometric parameters, the relative permittivity of the surrounding medium and the conductance at the bacteria surface have been fixed to decrease the number of degrees of freedom. As a first step, the model has been adjusted to fit the data obtained from the 5K *E.coli* bacteria strain, which is then used as a reference. Then variations on the model parameters have been studied in order to correlate the experimental data from the hha-hns double mutant bacteria.

From this study, it has been observed that there is a significant decrement in the cytoplasm conductivity of the double mutant, hha-hns, with and without conjugative plasmid R27, with respect that of the *E.coli* 5K. Moreover, the relative permittivity of the cytoplasm maintains its value for all the cases with only a small decrement with respect to the *E.coli 5K* value. A significant increment in the values of the rest of the dielectric parameters is observed when compared to the *E.coli* 5K case.

This characterization makes it is possible to define a conductivity medium region where it is possible to separate the double mutant bacteria from the non modified ones. However, the differences due to the presence of the conjugative plasmid R27 are not as clear as in the case of the mutant and non mutant comparative.

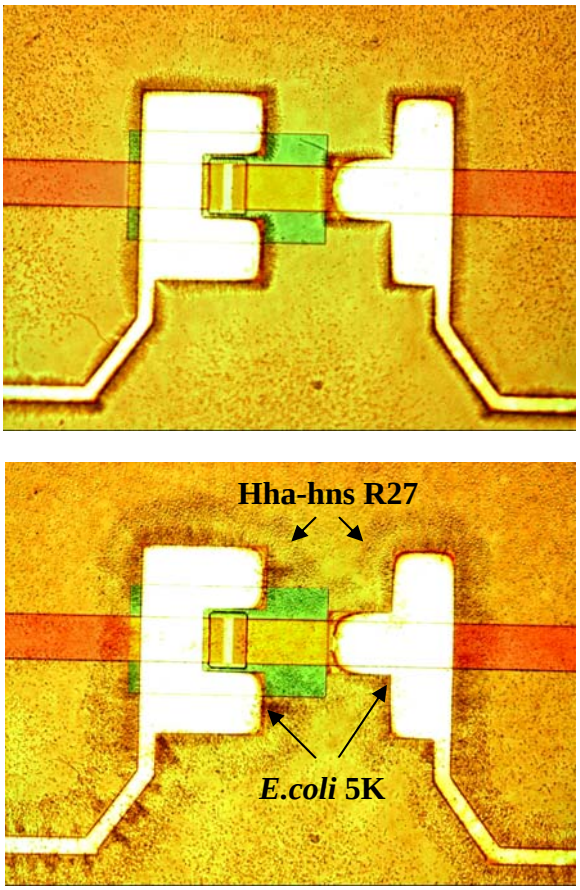

Figure 4: Bacteria separation by DEP: (top) both types of bacteria are trapped around the electrodes by p-DEP at 10MHz. (bottom) *E.coli* strain 5K is trapped at the electrodes by p-DEP whereas the hha-hns double mutants with plasmid R27 are repelled by n-DEP at 80MHz.

Conclusions and Future Work

The potential of conventional dielectrophoresis for the identification of differences in bacteria suspension at the level of genetic modification (double mutation) has been proven. This creates the possibility of distinguishing and separating such biological systems, advantageous for applications in the food industry and biomedical fields. Differences due to the conjugative plasmid must be comprehensively studied in order to examine the possibility of distinguishing bacteria with and without antibiotic resistance, which can be of interest for clinical analysis.

For the future, a microfluidic system is being developed for the containment of the different types of bacteria in different reservoirs which have previously been analysed and separated by DEP.

Acknowledgments

The authors would like to thank Mr. R. Casanella for his previous work on DEP, Mrs. M.C. Jaramillo for assistance in the preparation of samples and of Dr C. Mills for comments with respect to this work. Dr. A.

ISSN: 1727-1983

Errachid acknowledges support from the Ramon y Cajal program. Financial support for this work, provided by the Spanish Ministry of Science and Technology (MCYT) under Project TIC2001-3569-C02-01/02, is gratefully acknowledged.

References

[1] US-NLH National Institutes of Health, Internet site address: http://www.nlm.nih.gov/medlineplus/ bacterialinfections.html.

[2] MORGAN H., HUGHES M.P. AND GREEN N.G. (1999): 'Separation of submicron bioparticles by dielectrophoresis'. *Biophys. J.*,77, pp. 516– 525.

[3] CASANELLA R., SAMITIER J., ERRACHID A.,MADRID C., PAYTUBI S., A. JUÁREZ A. (2003): 'Aggregation profile characterisation in dielectrophoretic structures using bacteria and submicron latex particles'. *IEE Proc. Nanobiotechnol.*,150 (2), pp. 70-74.

[4] GERMISHUIZEN W.A., WÄLTI C., WIRTZ R., JOHNSTON M.B., PEPPER M., DAVIES A.G. AND MIDDELBERG A.P.J. (2003): 'Selective dielectrophoretic manipulation of surface-immobilized DNA molecules'. *Nanotechnol.*,14, pp. 896–902.

[5] SUEHIRO J., HATANO T., SHUTOU M. AND HARA M. (2005): 'Improvement of electric pulse shape for electropermeabilization - assisted dielectrophoretic impedance measurement for high sensitive bacteria detection'. *Sens. Act. B*,109(2), pp. 209-215.

[6] POHL. H.A. (1978): 'Dielectrophoresis'. Cambridge Univ. Press.

[7] PETHIG, R. (1979): 'Dielectric and Electronic Properties of Biological Materials'. Wiley & Sons, New York.

[8] JONES, T.B.(1995): 'Electromechanics of Particles'. Cambridge Univ. Press.

[9] CASTELLARNAU M., ZINE N., BAUSELLS J., MADRID C., JUÁREZ A., SAMITIER J. AND ERRACHID A. (2005): 'Integrated microanalytical system based on electrochemical detection and cell positioning'. *Mat. Sci. Eng. C-Bio S*. accepted.

[10] JUÁREZ A. AND GOEBEL W. (1984): 'Chromosomal mutation that affects excretion of hemolysin in *Escherichia coli*'. *J. Bacteriol.*,159, pp. 1083-1085.

[11] MIETCHEN D., SCHNELLE T., MÜLLER T., HAGEDORN R. AND FUHR G. (2002): 'Automated dielectric single cell spectroscopy—temperature dependence of electrorotation'. *J. Phys. D: Appl. Phys.*,35, pp.1258–1270.

[12] ZHENG L., BRODY J.P. AND BURKE P.J. (2004): 'Electronic manipulation of DNA, proteins, andnanoparticles for potential circuit assembly'. *Biosens. Bioelectron.*,20, pp. 606–619.

[13] ERMOLINA I. AND MORGAN H. (2005): 'The electrokinetic properties of latex particles: comparison of electrophoresis and dielectrophoresis'. *J. Colloid Interface Sci.*,285, pp. 419–428.

ISSN: 1727-1983