Separation of biological cells and bacteria by gradient electrodes

S. van den Driesche\textsuperscript{a*}, H. Zirath\textsuperscript{a}, D. Puchberger-Enengl\textsuperscript{a}, F. Iuliano\textsuperscript{b}, H. Wiesinger-Mayr\textsuperscript{c}, M. J. Vellekoop\textsuperscript{a}

\textsuperscript{a}Institute of Sensors and Actuator Systems, Vienna University of Technology, Gusshausstrasse 27-29, 1040, Vienna, Austria
\textsuperscript{b}Institute of Virology, Slovak Academy of Sciences, Dubravská Cesta 9, 84245, Bratislava, Slovakia
\textsuperscript{c}Health & Environment Department, Austrian Institute of Technology, Muthgasse 11, 1190, Vienna, Austria

Abstract

A method for the separation of biological cells and bacteria based on traveling wave dielectrophoresis (twDEP) is presented. The microfluidic chip consists of a structured PDMS layer on glass. Parallel electrodes with increasing width and gap size, positioned along the micro fluidic channel, were used to expose cells to a twDEP force perpendicular to a pressure driven flow. With this gradient electrode structure we show that successful separations of mixtures of bacteria contaminated cells into subpopulations of viable cells and bacteria are feasible.

© 2011 Published by Elsevier Ltd. Open access under CC BY-NC-ND license.

Keywords: Cell separation, microfluidics, traveling wave dielectrophoresis

1. Introduction

Suspended-growing viable biological cells are difficult to separate from cell culture debris and bacterial contamination. A way to separate suspended viable cells is by using field flow fractionating [1], fluorescence activated cell sorting [2] or magnetic activated cell sorting [3], requiring a particle density difference or expensive elaborate labeling steps. A phenomenon that does not require labeling for separation is dielectrophoresis, defined as the motion of polarizable particles exposed to a non-uniform electric field. To the best of our knowledge, twDEP is the only DEP based method allowing an efficient biological cell and bacteria separation. In previous work we have shown a continuous cell-from-cell separation based on twDEP without gradient electrodes, which allowed cell-cell separation but did not

\* Corresponding author. Tel.: +43-1-58801-36670; fax: +43-1-58801-36699.
E-mail address: sander.driesche@tuwien.ac.at
result in a single position focusing of bacteria [4, 5]. In this contribution, a bacteria barrier effect created by gradient electrodes was used to separate the viable cells and bacteria.

2. Separation principle

The basic twDEP equation approximation for first order dipoles is as follows:

\[ F_{\text{twDEP}} = -\frac{4\pi^2\varepsilon_r^3}{\lambda} \Im[f_m(\omega)] E_{\text{rms}} \quad \text{with} \quad \Im[f_m(\omega)] \in [-0.75...0.75] \quad (1) \]

where \( \varepsilon_r \) is the permittivity of the surrounding medium, \( r \) the particle radius, \( \lambda \) the wavelength of the traveling-field (distance between four electrodes when a 0°, 90°, 180°, and 270° phase shift is used), \( \Im[f_m(\omega)] \) the imaginary part of the Clausius-Mossotti factor, \( \omega \) the angular frequency, and \( E_{\text{rms}}^2 \) the electric field squared.

By increasing the width and gap size (increasing \( \lambda \) in Eq. 1, Fig. 1) of parallel electrodes positioned along the microfluidic channel, the twDEP force acting on the particles decreases (in the direction perpendicular of the electrodes of increasing width).

The experimental results presented in previous work, where bacteria were aligned at distinct positions and did not cross the 20 \( \mu \)m wide electrodes (due to the large electrode gap, under the set conditions), show that bacteria could be separated from the sample mix by an optimized electrode array. To investigate whether it is feasible to also separate bacteria from a sample mixture a chip was designed and realized consisting of an electrode array of different widths (increasing from 7.5 \( \mu \)m, 11.25 \( \mu \)m, 15 \( \mu \)m, and 20 \( \mu \)m) positioned along the channel (Fig. 1). Cells and bacteria exposed to the traveling electric field were introduced at the lower y-region and were experiencing a negative DEP force in the direction towards the higher z-region away from the electrodes (Fig. 2A). The cells also experienced a twDEP force antiparallel to the field. By increasing the electrode-width the electric field strength above the electrodes decreases, creating gaps in the traveling electric field (Fig. 2B). Wider electrodes result in a larger gap. When the gap is large enough, resulting in a twDEP force too low to push bacteria over, a barrier is created. For larger cells these gaps are easier to cross (see Eq. 1) allowing them to move towards higher y-regions.
Unaffected, non-viable cells and cell debris will leave the channel in the same y-region as they were introduced (Fig. 2).

The proposed separation method over an increasing electrode width was investigated on a sample mixture consisting of *Saccharomyces cerevisiae* cells and *Lactobacillus casei* bacteria.

3. Results and Discussion

The frequency and voltage of the traveling electric field were selected to push the viable *S. cerevisiae* and bacteria against the traveling field. At the same time the DEP force at the selected frequency was negative, pushing the cells away from the electrodes towards the channel ceiling (which prevents the sticking of cells at the electrodes). The biological mix was suspended in sucrose (10 %w/w) dextrose (2 %w/w) buffer with the conductivity set to 30 mS/m by adding phosphate buffered saline. A successful separation of the viable *S. cerevisiae* cells and *L. casei* bacteria from the mix is depicted in Fig. 3. The gap created above the first 15 μm wide electrode functioned as a bacteria barrier (Fig. 2B, red dashed line). The traveling electric field strength above this electrode was too low to create a twDEP force pushing the bacteria over (Eq. 1). For the larger *S. cerevisiae* cells, the twDEP force was large enough to push the cells across the electrode.

4. Conclusions

With parallel twDEP electrodes of increasing width and gap size positioned along the microfluidic channel, we are able to create a barrier bacteria cannot cross. Our experiments show that a mixture of viable *S. cerevisiae* cells and *L. casei* bacteria exposed to a traveling electric field created above gradient electrodes could be separated into distinct populations. The viable *S. cerevisiae* cells moved across the entire width of the separation channel while *L. casei* bacteria only moved up to the channel width position between the 11.25 μm and 15 μm width electrodes.
Fig. 3: A sample mix consisting of *S. cerevisiae* and *L. casei* bacteria, introduced over the whole width of the micro fluidic channel, was dragged through the channel by a pressure driven flow and exposed to a traveling-electric-field of 3.5 V\textit{rms} at 180 kHz. Viable *S. cerevisiae* cells moving in the micro-fluidic channel (highlighted by arrows) experienced a twDEP force (directed against the travelling field) perpendicular to the flow direction, left the separation channel at a distinct position. *L. casei* bacteria that were introduced in the channel region above the 7.5 and 11.25 μm width electrodes also experienced a twDEP force (directed against the traveling field) perpendicular to the flow, but were blocked (and therefore focused) by the first 15μm width electrode due to the relative large electric field strength gap above this electrode (compared to the small bacteria size).

**Acknowledgements**

This project is a part of an EU Marie Curie Research Training Network (MRTN): On-Chip Cell Handling and Analysis, CellCheck. Proj. no. MRTN-CT-2006-035854. For the fabrication of the devices, the authors would like to acknowledge E. Svasek and P. Svasek from the Sensor Technology Lab at ISAS and the Center for Micro- and Nanostructures (ZMNS), TU Vienna.

**References**

[1] B. Roda, A. Zattoni, P. Reschiglian, M. H. Moon, M. Mirasoli, E. Michelini, and A. Roda, "Field-flow fractionation in bioanalysis: A review of recent trends," *Anal. Chim. Acta*, vol. 635, pp. 132-143, Mar 2009.

[2] S. H. Cho, C. H. Chen, F. S. Tsai, J. M. Godin, and Y.-H. Lo, "Human mammalian cell sorting using a highly integrated micro-fabricated fluorescence-activated cell sorter (FACS)," *Lab Chip*, vol. 10, pp. 1567-1573, Jun 2010.

[3] I. Safarik and M. Safarikova, "Use of magnetic techniques for the isolation of cells," *J. Chromatogr. B*, vol. 722, pp. 33-53, 1999.

[4] S. van den Driesche, V. Rao, D. Puchberger, W. Witarski, and M. J. Vellekoop, "Continuous separation of viable cells by travelling wave dielectrophoresis," in *Procedia Engineering* 5, Linz, Austria, 2010, pp. 41–44.

[5] S. van den Driesche, V. Rao, D. Puchberger-Enengl, W. Witarski, and M. J. Vellekoop, "Continuous cell from cell separation by travelling wave dielectrophoresis," *Sens. Actuators B*, 2011. DOI:10.1016/j.snb.2011.01.012