Cell Separation in a Continuous Flow by Traveling Wave Dielectrophoresis

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In this contribution we present a microfluidic chip for the continuous and label-free separation of cells. Strip electrodes produce a traveling electric field perpendicular to the pressure driven flow. Viable cells are deflected parallel to the field by traveling wave dielectrophoresis (twDEP) according to their volume and dielectric properties. With the present device we have successfully separated viable *Saccharomyces cerevisiae* and *Jurkat* T-cells from debris, non-viable cells *and Lactobacillus casei*.

Introduction

Common techniques to separate suspension-grown cells, such as Fluorescent Activated Cell Sorting (FACS), require elaborate protocols and equipment. Dielectrophoresis (DEP) has widely been applied for the label-free separation of particles and biological cells [1]. Traveling wave dielectrophoresis is the linear motion of particles and cells induced by a traveling electric field that is produced by multi-phase excited microelectrodes (Fig. 1). In contrast to conventional dielectrophoresis, twDEP does not require a nonuniform electric field. Therefore, the force does not decay with distance and cells can be moved with a better efficiency [2]. The force on a particle in a traveling electric field is given by

$$\vec{F}_{twDEP} = -\frac{4\pi^2 r^3 \varepsilon_m}{\lambda} \Im\{K(\omega)\} E^2_{rms} \tag{1}$$

where r is the particle radius, ε_m the medium permittivity, E the field magnitude, λ the electrode distance and $\Im\{K(\omega)\}$ the imaginary part of the Clausius-Mosotti factor.

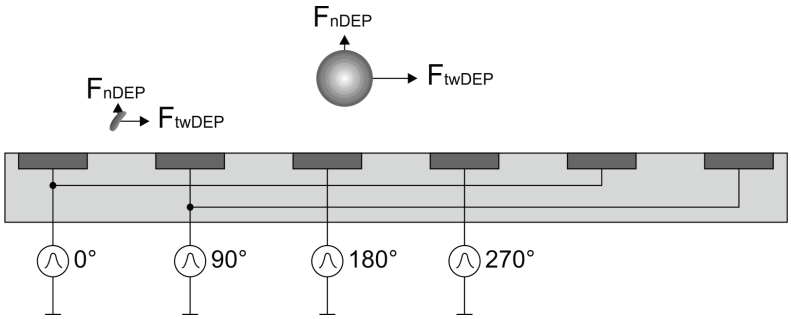

Fig.1: Forces on suspended cells of different size exposed to a traveling field. The cells are levitated by negative Dielectrophoresis ($\Re\{K(\omega)\} < 0$) and propelled across the electrodes according to the imaginary part of the Clausius-Mosotti $\Im\{K(\omega)\}$ [3].

Materials and Methods

The medium for cell experiments consisted of 10% (w/w) sucrose (Sigma S9378) and 2% (w/w) dextrose (Sigma D9559) in deionized water. The conductivity was set to 40 mS/m by adding PBS. *Jurkat* T-lymphocytes were cultivated at 37 °C in a DMEM medium containing 4.5 g/l glucose, 2 mM L-glutamine, 10% fetal calf serum and antibiotics (100 units/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml amphotericin B). Baker's yeast (*Saccharomyces cerevisiae*) was obtained fresh from a supermarket. Contaminated samples were prepared by adding *L. casei* bacteria (DSM20011) to the cells in suspension. Prior the experiments the cells were washed twice and resuspended in DEP medium.

The fabricated chip and its operation principle are illustrated in Fig. 2. A set of parallel platinum electrodes of 20 µm width and gap size was fabricated on a glass wafer by a lift-off process. Holes for fluidic access were drilled through the glass substrate. The 200 µm wide and 30 µm high channel was fabricated by soft-lithography in PDMS and placed on top of the electrodes.

For the continuous experiments the cell samples are introduced into the chip by a syringe pump (KD Scientific 210P). A sheath flow from the second inlet is used to focus the cells to one side of the channel (Fig. 2). Two signal generators (Agilent 33220A) and broadband transducers are utilized to produce the decoupled and phase-shifted excitation voltage. According to the chosen frequency of the traveling field the target cells are dragged across the channel and leave the device separated from contamination and debris.

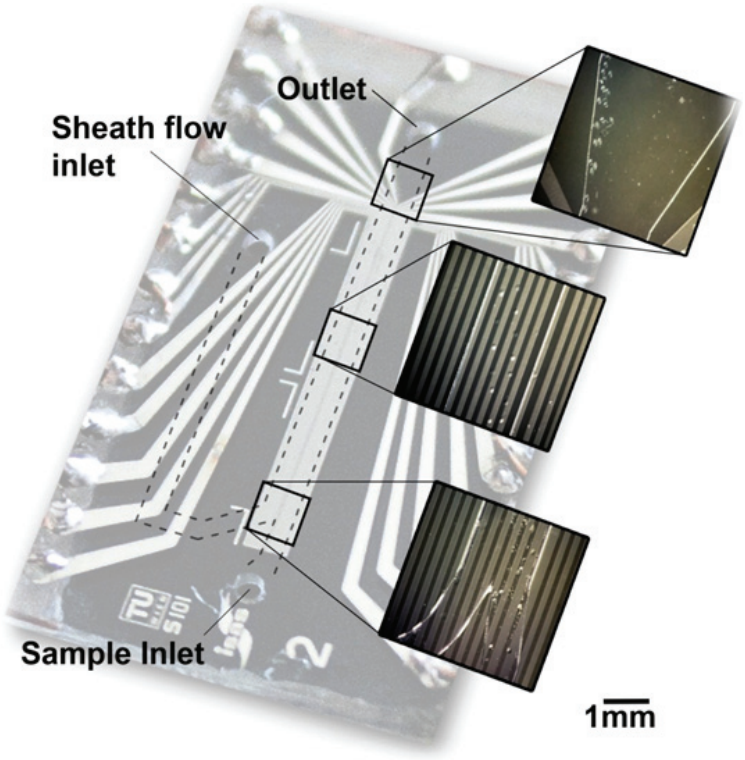

Fig. 2: Separation chip: The sample stream is focused to one side and target cells are moved across the electrodes towards the channel wall.

Results and Discussion

To determine the optimal frequency for cell-cell separation the imaginary parts of the Clausius-Mosotti factor $\Im\{K(\omega)\}$ of the three cell types were evaluated by electrorotation (ROT).

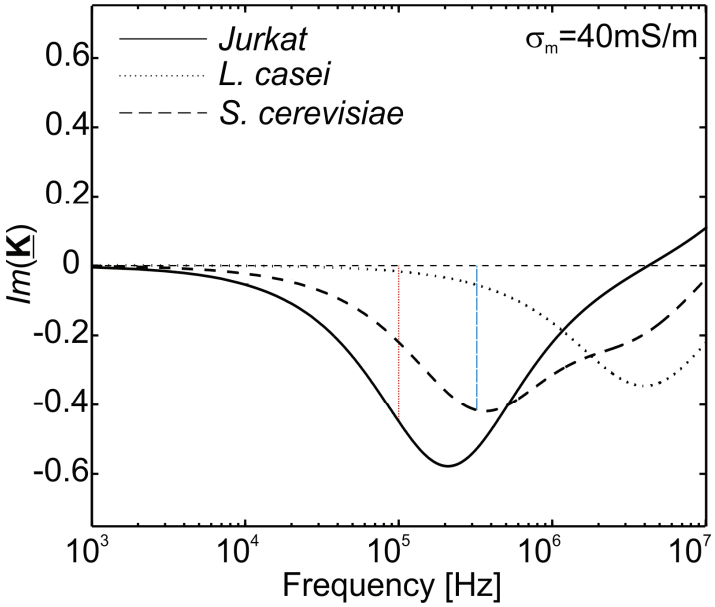

Fig. 3: Imaginary parts of Clausius-Mosotti factor of *Jurkat* T-cells (~14µm), *Saccharomyces cerevisiae* (~5µm) and *L. casei* (~1x3µm).

A quadruple electrode configuration was used to measure cell rotations per second with varying frequency. Cell models with multiple shells, representing the cytoplasm, cell membrane and cell wall, were fitted to the experimental data [4]. The modeled frequency responses of $\Im\{K(\omega)\}$ for the used cell types are plotted in Fig. 3. The vertical lines indicate the frequencies used for separation experiments. According to Eq.1 the differences of $\Im\{K(\omega)\}$ at these frequencies in combination with the distinct cell radii suggest a great difference of the resulting twDEP force and enable efficient separation.

Results of the separation experiments are illustrated by cell counts at the chip outlet shown in Fig. 4. The cell samples were introduced with a flow rate of 0.1 µl/min and focused by a sheath flow of 0.15 µl/min. Without an applied voltage the cells left the device as they were introduced, only on one side of the channel (DEP off diagrams in Fig. 4). After applying a voltage, viable cells were forced towards the other side of the channel. Non-viable cells did not experience a twDEP force due to disruption of the cell membrane. Furthermore, the *L. casei* bacteria were not affected by traveling wave DEP and left the device at local field minima above the electrodes in the range of 0 – 100 µm. Altogether 86.5% of the *Jurkat* cells were found in the 170 to 200 µm region. This percentage corresponds to a viability test with trypan blue staining prior the experiments that revealed a cell viability of 90%. In experiments with yeast 87% left the channel in the 185 to 200 µm region. Unaffected cells left at the local field minimum above the first electrode, suggesting to decrease the electrode width for smaller cells.

Conclusion

Traveling wave dielectrophoresis has been shown to yield an efficient, label-free purification of biological cells from a contaminated sample solution. Only rough sample fo-

cusing is required and the separation is robust against flow rate fluctuations. Besides separation the presented device also enables viability studies since only cells with intact membranes are affected by the twDEP force.

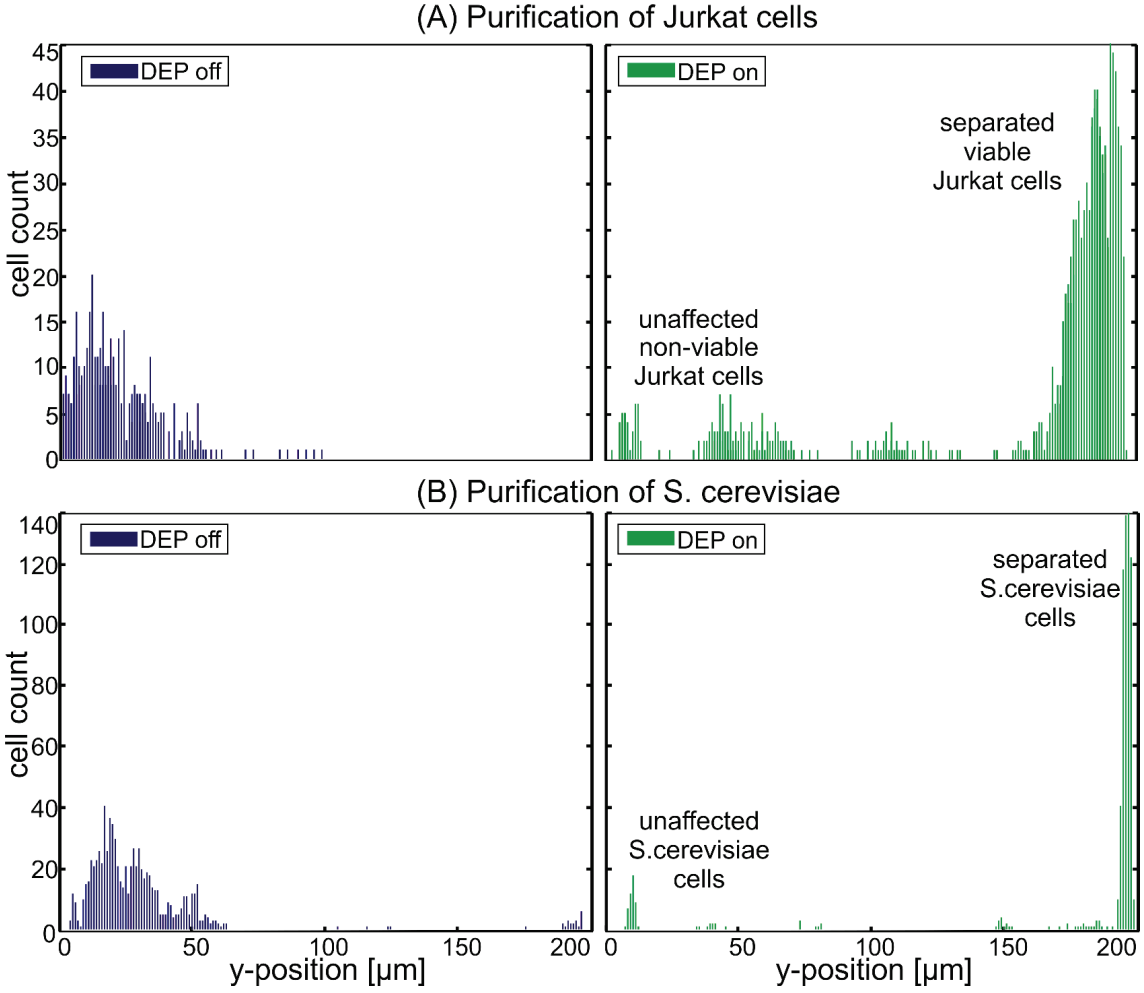

Fig. 4: Histograms of the separation results (total flow rate 0.25 µL/min, medium conductivity 40 mS/m). (A): Viable *Jurkat* cells separated from cell culture debris containing non-viable cells and *L. casei* (voltage 3.5 Vrms, 100 kHz). (B): *S. cerevisiae* cells separated from cell culture debris containing non-viable cells and *L. casei* bacteria (voltage 5 Vrms, 350 kHz).

Acknowledgements

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References

[1] R. Pethig: "Review Article-Dielectrophoresis: Status of the theory, technology, and applications", Biomicrofluidics, 2010 Jun 29;4 (2)

[2] S. van den Driesche, V. Rao, D. Puchberger-Enengl, W. Witarski, M. J. Vellekoop: “Continuous cell from cell separation by traveling wave dielectrophoresis”, Sensors and Actuators B: Chemical, 2011, Article in Press

[3] T. B. Jones: “Basic Theory of Dielectrophoresis and Electrorotation” IEEE Eng Med Biol Mag. 2003 Nov-Dec;22(6): 33-42

[4] P. Gascoyne, F.F. Becker, X.-B. Wang: “Numerical analysis of the influence of experimental conditions on the accuracy of dielectric parameters derived from electrorotation measurements”, Bioelectrochemistry and Bioenergetics, Vol. 36, 2, 1995, 115-125