MICROFLUIDIC DEVICE FOR CONTINUOUS DICELECTROPHORETIC SEPARATION OF CELLS IN DIVISION

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ABSTRACT

This paper reports on a novel method for on-chip continuous separation of dividing and non-dividing cells based on differences in their dielectric properties. By means of two opposite dielectrophoretic force fields at multiple frequencies, the two populations of cells flowing through the microfluidic device are focused towards distinct equilibrium positions, which can be correlated to their cell cycle.

KEYWORDS: cell cycle, cell separation, dielectrophoresis, cell synchronization

INTRODUCTION

Cell culture synchronization provides cells that are at the same phase within their cell cycle. The homogeneity of the cell cultures has a significant importance in biotechnology to develop and improve particular processes such as protein synthesis, as well as in research fields such as drug discovery to evaluate a biological process and assess its dynamics. We propose to address cell synchronization by applying our continuous-flow cell separation method. This microfluidic device has been previously used to separate a mixture of yeast cells into pure fractions of viable and non-viable yeast cells [1, 2]. It was also utilized to increase the infection rate of an infected red blood cell population up to 50% which was an important issue in the investigation of the parasite life-cycle. Our versatile separation method is now applied to the synchronization of cell culture by isolating the cells that are dividing. Compared to previously reported DEP-based continuous cell separation [3, 4], our method makes use of opposed DEP forces at multiple frequencies to create a position of equilibrium as a function of the dielectric properties of the cells.

EXPERIMENTAL

The two-steps microfabrication process of the microfluidic device is shown in Fig. 1a. Platinum electrodes are first patterned onto a Pyrex substrate by lift-off and microchannels are then structured in SU-8 by photolithography (Fig. 1a). The design consists of a central channel through which cells flow with dead-end chambers perpendicular to it where the metal electrodes are deposited (Fig 1b). The electrode array comprises 2x15 electrodes along the sidewalls of this central channel. An electric field is generated on each side by applying successively positive and negative potentials to the electrodes. The insulator guides the field lines creating local non-uniformities in the central channel. The non-uniform electric field induces a dipole on the flowing cells producing dielectrophoresis. The electrode array generates two opposite DEP-force fields (\(F_{\text{DEP,1}}\) and \(F_{\text{DEP,2}}\) in Fig. 2) which focus the cells towards...
an equilibrium position according to their dielectric properties. The superposition of two electric signals on one side of the electrode array is used to generate DEP-forces at multiple frequencies (Fig 2) enabling cell separation [5].

**Figure 1.** (a) Process flow of the microfabrication. (b) SEM image of the central channel along which metal electrodes are deposited.

**RESULTS AND DISCUSSION**

Yeast cell was chosen as a cell model due to its short cell-division cycle. Fig. 3 reports the output positions of cells after separation as a function of the scanning frequency ($f_{\text{scan}}$ in Fig 2) while the other parameters remain constant. It can be seen that the separation efficiency (the difference between the mean output positions) is the highest in the frequency range between 1 and 1.5 MHz.

**Figure 2.** Schematic of the separation concept. Electric signals on both sides generate two opposite DEP-force fields ($F_{\text{DEP,1}}$ and $F_{\text{DEP,2}}$).

**Figure 3.** Output positions of the two populations of normal cells and cells in division after separation as a function of the frequency of the scanning signal.
Cells in division ended up at more negative output positions than non-dividing cells, which means that they are more attracted by the pDEP. This is also illustrated in Fig 4a for an $f_{\text{scan}} = 1.5 \, \text{MHz}$. The corresponding histogram of the output positions for the two subpopulations, dividing and non-dividing yeasts, is shown in Fig 4b.

**Figure 4:** (a) Picture of flowing cells downstream the separation structure; cells in division are strongly attracted towards the left side where the pDEP is applied (b) Histogram of the output positions of the two populations after separation at the scanning frequency of $1.5 \, \text{MHz}$.

**CONCLUSIONS**

With this separation method a homogeneous and synchronized fraction of dividing cells is obtained. This makes it a powerful tool in cell-division cycle studies and protein expression research. We are currently working on the quantification of the synchronization by means of specific fluorescent markers and the correlation of the measured dielectric properties to the cell cycle.

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