CONTINUOUS-FLOW CELL TRAPPING AND HYBRIDOMA-CELL PRODUCTION ON CHIP USING LIQUID ELECTRODES

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ABSTRACT

This paper reports on a new microfluidic chip designed for cell electrofusion. A pair of cells is brought into close contact by means of opposite dielectrophoretic forces and they are subsequently fused by applying a short electrical pulse.

KEYWORDS: cell fusion, dielectrophoresis, liquid electrodes.

INTRODUCTION

Creation of hybridoma cell lines via electrofusion is a powerful method for the production of antibodies. While very low fusion yield is reached with macroscopic methods, a few examples of efficient and controlled cell fusion using a microfluidic approach have been shown [1,2]. With our microfluidic chip we intend to improve the reproducibility and reliability of cell fusion.

The microfluidic device consists of a network of photopatterned channels in SU8 with Ti-Pt electrodes previously structured by a lift-off process (Fig 1). The chip is reversibly sealed by a molded part in PDMS containing embedded liquid reservoirs and the full device is mounted into a custom-made fluidic and electrical interface (Fig 1). It relies on the concept of liquid electrodes for dielectrophoresis [3].

Figure 1. (a) Drawing of the microfluidic chip design (b) SEM image of the fusion chamber along which metal electrodes are deposited

EXPERIMENTAL

Two inlet channels, one for each cell type, join to form the central channel where the cell fusion chamber is located. To provide the fusion chamber with cells only on demand, each inlet channel splits in two branches, one towards the fusion chamber and the other towards the waste. An array of liquid electrodes is patterned along the channel, in the so called cell deviation region, before the bifurcation (Fig 2a). When
the electrodes are activated by an AC voltage of sufficient magnitude (same voltage but opposite phase on consecutive electrodes), each cell experiences a negative dielectrophoretic force (perpendicular to the fluid direction) and is deflected towards the waste [4]. Deactivating the electrodes lets cells flow toward the fusion chamber. The cell fusion chamber has a number of electrodes patterned on both sides of the channel. Four of them allow the trapping of the cells by negative dielectrophoresis. Once two cells are trapped, a short square pulse is applied to another pair of electrodes making both cells fuse (Fig 2b).

![Figure 2](image)

**Figure 2.** (a) Schematic of the cell deviation region in the microfluidic chip. AC signals generate a DEP-force field in the flowing cells directing them towards the waste. (b) Schematic of the fusion chamber. Electric signals on both sides generate two opposite DEP-forces Cells flowing downstream get trapped and fused by a short electrical pulse.

**RESULTS AND DISCUSSION**

For the experiments, T-lymphocytes (EG7) cells suspended in a low conductivity phosphate buffer solution (50 mS/m) are used. A 100 kHz 20V_{pp} signal was applied to the deviation electrodes in order to drive the cells towards the waste (Fig.3).

![Figure 3](image)

**Figure 3.** (a) AC signal off: cells flowing towards fusion chamber and waste (b) AC signal on: a 100 kHz 20V_{pp} signal was applied to the electrodes to direct cells towards the waste.

For the cells being trapped in the fusion chamber a 10V_{pp} signal at 200 kHz is applied. Viability test of the trapped cells with Propidium Iodide (PI) showed that the DEP trap is harmless for the cells. To induce cell fusion different pulse configurations have been tested; a train of short pulses (three pulses of 100 μs, 1s between pulses) and high electric field (3kVcm\(^{-1}\)) or longer pulses (4 ms) with lower electric field (0.9 kVcm\(^{-1}\),Fig 4).
CONCLUSIONS

We have developed a new microfluidic device capable of trapping and fusing a pair of cells in a continuous flow. We are currently evaluating the optimum fusion parameters and the viability of the cell fusion products by means of specific fluorescent markers. We foresee a great potential for this type of devices as tool for cell electrofusion and creation of hybridoma cell lines, which is essential in the production of monoclonal antibodies for immunological related diseases and vaccines in cancer immunotherapy.

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REFERENCES


