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A MICROFLUIDIC BIOCHIP DEDICATED TO HIGHLY PARALLELIZED ELECTROFUSION

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ABSTRACT

This paper deals with the development of a biochip dedicated to fusion between two types of cells, in a highly parallelized way. The fusion process is initiated by an electric field pulse applied to cells paired in fluidic traps. The paper will present different strategies for the capture and pairing of cells prior to the electrofusion, combining fluidic and electrical forces. The design is highly parallelized in order to fulfill our objective which is the high throughput production of hybrid cells.

The microfabrication process is described. Experimental results confirm the efficiency of the proposed device for cell trapping, pairing and fusion.

KEY WORDS: Microfluidics, Biochip, Electrofusion, Cell trapping

INTRODUCTION

The electrofusion between a dendritic and a cancerous cell is one of the new techniques investigated for immunotherapy of cancer. Hybridomas produced massively might be reinjected *in vivo* as a vaccine [1]. Nowadays, electrofusion being performed in macroscopic chambers suffer of a poor efficiency. The miniaturized approach developed in this paper provides new avenues to enhance and control the different electrofusion steps, and go towards the high throughput production of hybrid cells.

To achieve parallel fusions on chip, three steps have to be integrated i) the trapping of cells flowing in the microfluidic channel ii) their pairing prior to fusion, iii) the electrofusion operation while cells are maintained at close contact. When the transmembrane voltage reaches 0.3V, membranes are permeabilized, and finally mixed leading to the formation of a hybrid cell including the cell content of the two mother cells.

DESIGN

The design of the proposed biochip is derived from a device originally devoted to the mixing of droplets [2]. The pairing is performed in three steps i) cells type A are captured in location 1 thanks to the flow as shown on figure 1 ii) the flow is reversed, leading cells type A to move towards the facing fusion chamber in location 2 (see figure 1) iii) then cells type B are injected to be placed in location 2. Gold electrodes placed at both sides of the fusion chamber permit to apply the electric field (see figure 2) necessary to put into contact the cells of type A and B, and finally induce their fusion through an electropermeabilization pulse.

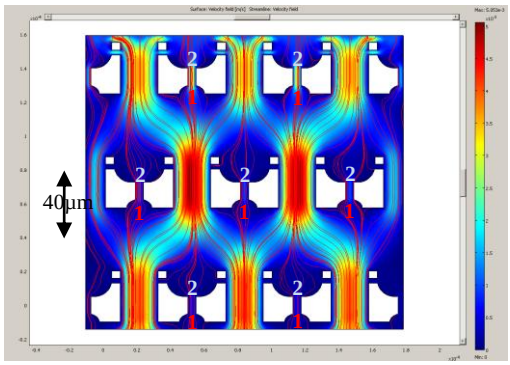

Figure 1: Comsol© simulation of the flow induced through the fluidic traps

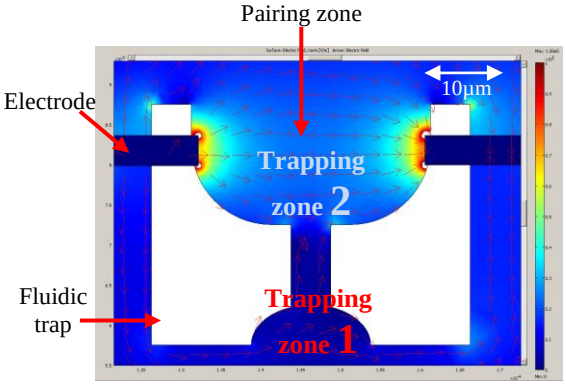

Figure 2: Comsol© simulation of the Electric field within the trap zone

PROCESS OF FABRICATION

The microfluidic chip fabrication is based on photolithography process. Glass is used as substrate for the device in order to ensure the transparency that will permit the real time monitoring of the fusion operation. Gold is chosen as material for the electrodes, to insure the biocompatibility of the device. In classical microfluidic devices, structured PDMS composes the channels [3]. But in this method, a good alignment with the electrodes is not possible. To ensure

proper alignment, the microfluidic structures of the biochip presented (channels and fluidic traps) are fabricated using SU8-2025 (t= 25µm) thick photosensitive resist (figure 3).

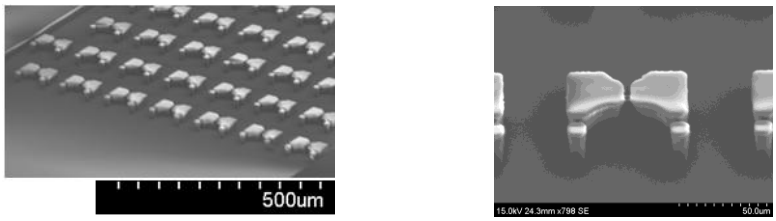

Figure 3: SEM view of the microfluidic traps

The packaging of the biochip is performed thanks to the bonding of a PDMS cover on the top of the SU8 fluidic structure. To do so, silanization of the photoresist after its hard baking (2 hours at 175°C) is performed in liquid phase. After the silanization, the two surfaces (SU8 and PDMS) are activated with O_2 plasma before being bonded on a hot plate. The microfabricated device is shown on figure 4.

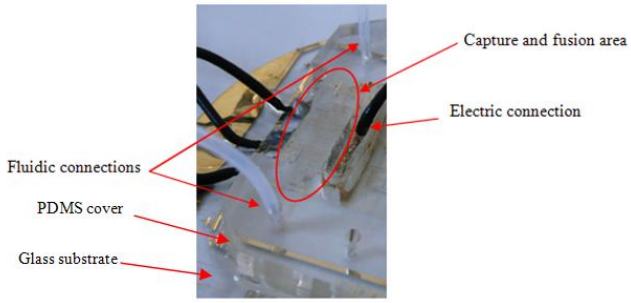

Figure 4: Packaged and connected electrofusion biochip

RESULTS

B16F10 (mouse melanoma cells) with a concentration of 10^6 cells/ml in a Minimum Essential Media were used for the biological tests. In these initial tests a single cell type was used to analyze cells' placement in the microfluidic traps. The cells were injected in the biochip using a syringe pump and were paired using different patterned traps according to the 3 steps previously described (figures 5 and 6). Electric pulses were then applied using a Cliniporator™ generator (IGEA, Carpi, Italy) exposing the cells to 100µs pulses of about 2kV/cm amplitude. This demonstrates that a highly parallelized trapping and pairing of cells can be done prior to electrofusion on a microfluidic device. Analysis of the fusion efficiency and quality are underway.

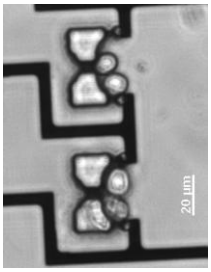

Figure 5: Result of the microfluidic trapping of cells

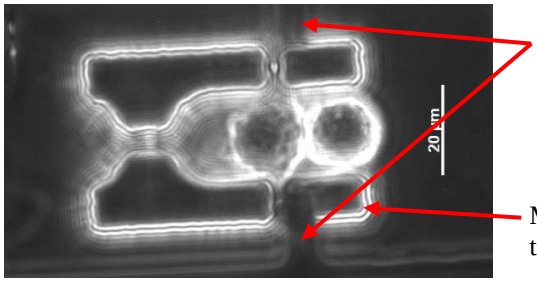

Figure 6: Paired cells under the application of electric pulses for the fusion

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