High Density Cell Electrofusion on Chip using an Array of Non-connected Metallic Pads

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Abstract: Cell fusion consists on creating a hybridoma cell containing the genetic properties of the progenitor cells. It can be performed chemically or electrically. The latter method, called Electrofusion, is a more efficient way to create hybrid cells investigated for antibody production or cancer immunotherapy. To envision this application, a high amount of hybrid cells is needed. This work presents an original design for high density electrofusion on chip. The structure consists of an array of non-connected electroplated gold pads patterned between two connected electrodes. While applying a Voltage on the connected electrodes, the Electric field is disturbed around the gold pads inducing a Dielectrophoretic Force on cells used to trap and pair them. When cells are paired, Electric pulses are applied to induce electrofusion. The absence of wire connections on the pads permits the high density trapping and electrofusion. Successful alignment and electrofusion of murine melanoma cells with this structure are demonstrated.

1 INTRODUCTION

Cell fusion is a method to generate a hybrid cell which combines specific properties of its progenitor cells. While cell fusion has been developed for antibody production (Köhler and Milstein, 1975), it is now also investigated for cancer immunotherapy (Sukhorukov et al., 2006) and reprogramming of somatic cells (Tada et al., 2001). There are different methods for cell fusion, such as biological (Okada, 1958), chemical (Pontecorvo, 1975) and electric pulse mediations (electrofusion). For electrofusion, the operation is simple and the system is free of chemical or genetic contaminations. Combined to the high fusion efficiency (Skelley et al., 2009)), this makes the method widely used. Therefore, the most used method for electrofusion consists on using an electroporation cuvette composed of two facing electrodes with 1 to 4 mm distance or ellipsoidal ones with 200µm inter-electrodes distance (Eppendorf). In these cuvettes, there is no possible placing of cells and the yield of one-to-one fusion cells is very low (20% fusion rate including multiple cell fusion (Zimmermann et al., 2006)). The use of small biodevices is investigated since 1989 (Masuda et al., 1989) to solve this problem by a primary step of placing cells. Different strategies for cell trapping and pairing were presented such as fluidic (Skelley et al., 2009) or electric using a Dielectrophoretic force (Masuda et al. 1989); (Techaumnat et al., 2008); (Kirschbaum et al., 2012).

Another issue in the development of biodevices for electrofusion is the yield. Indeed, a large amount of hybridomas is needed to make an injection, but in the classic microfabricated structures, the density is limited because of the electric connections.

In this work, we present a novel structure dedicated to electrofusion on chip with high density capability. The structure involves an array of non-connected micro-size electroplated gold pads, positioned between two electrodes, which induce a specific electric field topology. The absence of wiring, and connections, as gold structures are not powered, permits the high density arraying which is necessary when the high throughput is envisioned for the electrofusion. The successful self-alignment of cells in this array, thanks to DiElectroPhoretic (DEP) forces, followed by the application of electrofusion pulses is demonstrated.

In this paper, we first introduce the structure design with an FEM simulation of the electric field.

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Secondly, the fabrication process of the biochip and the preparation of cells are presented. Finally, experiments for cell trapping and electrofusion are described in the last sections.

2 MATERIALS AND METHODS

In this work, electrofusion on chip is based on the use of non connected high conductive materials (gold) to modify the electric field topology between two electrodes (Figure 1). This enables cells alignment and pairing between arrayed gold structures with assistance of DEP force, which is necessary before initiating the cell electrofusion protocol. To create the new design, a 3D simulation of the Electric Field and DEP in the structure had been achieved using a finite element method (AC/DC module of COMSOL Multiphysics ©). The result (figures 2 and 3) shows that with the appropriate conditions, cells are attracted to the area between the facing pads.

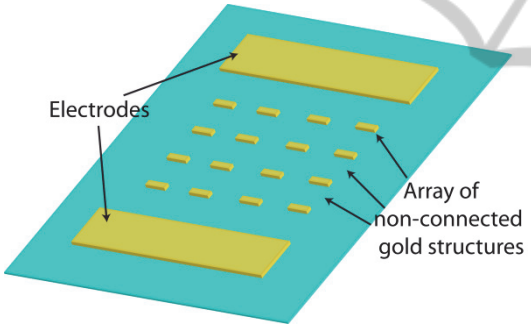

Figure 1: Schematic view of the chip developed showing electrodes generating electric field and gold structures for DEP effect.

2.1 Structure Modeling

Using the AC/DC module of COMSOL Multiphysics, we simulated a structure composed of an array of 6x4 non-connected metallic pads (10 µm x 40 µm size separated by 50 µm distance) between two electrodes (410 µm distance). The metal is 5.5 µm thick gold. The structure was immersed in 25 µm height low conductivity medium (0.03 S/m) and we applied 50 V on the connected electrodes.

As shown in figure 2a and 2b, the maximum electric field (E) values are between the gold pads (parallel to E) close to the edges, while the minima are above the pads, in the middle and on the edges perpendicular to the electric field (Figure 3a).

The electric field gradient obtained is used to trap cells. The gap between pads will then constitute the fusion zone. Thus, this gap is calculated to be equivalent to the size of two cells.

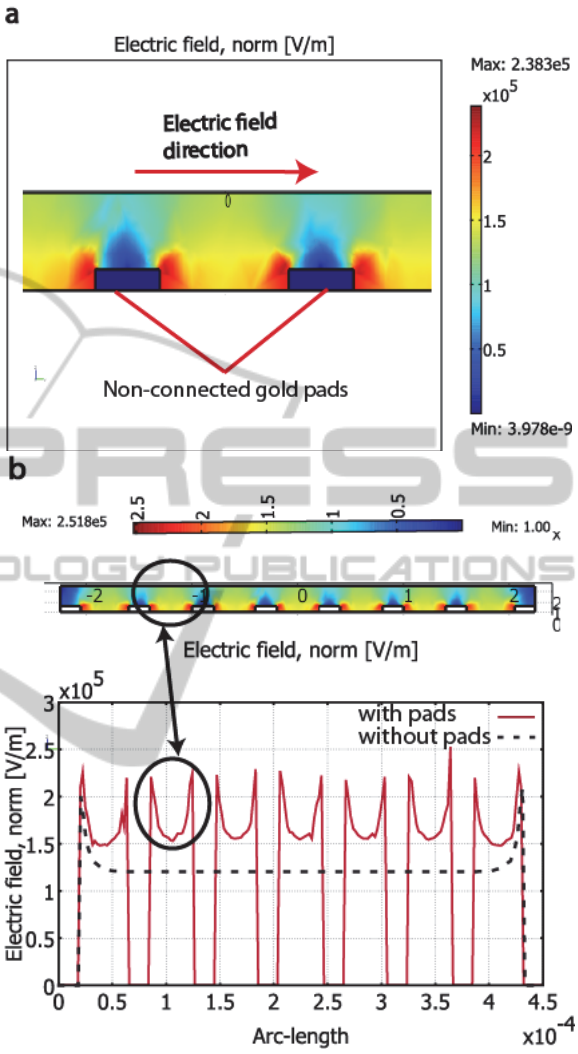

Figure 2: a.) Cut view of the 3D simulated structure showing electric field topology (color) b.) Electric field distribution along a raw of pads (continuous curve) and without pads (discontinuous curve)

Figure 2b shows that without the presence of the non-connected pads, the area between the electrodes presents a very homogeneous E but lowered to 1.2 kV/cm. If we apply the same voltage with the presence of the pads, the electric field in the fusion zone is increased to 1.5 kV/cm.

Due to the non-homogeneity of the electric field induced by the presence of the pads, insulating particles (as cells) would experience Dielectrophoresis in this structure. If we want to trap cells in the fusion zones, we can apply a sinusoidal

wave at a frequency inducing positive Dielectrophoresis (particles attracted by high E areas). Indeed, as shown by arrows in figure 3, if cells are in low E field areas, they will be pushed to the fusion zone (high E).

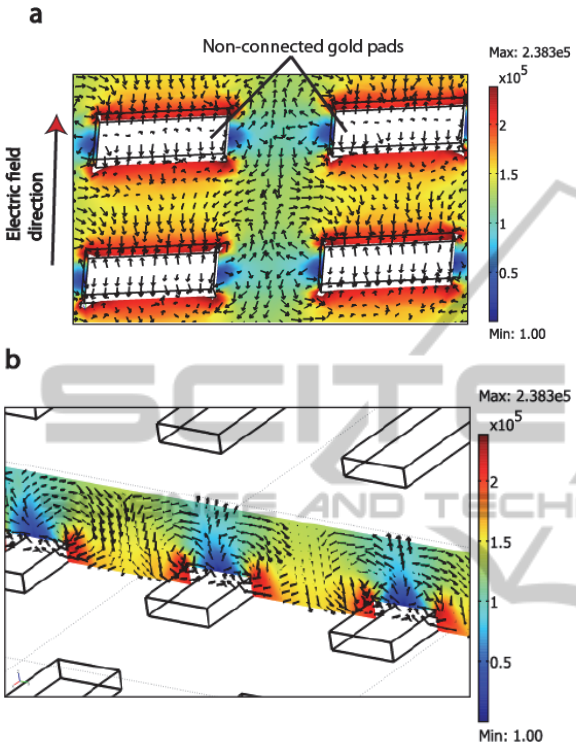

Figure 3: 3D simulated structure showing electric field topology (color) and DEP direction (arrow, case positive).

Therefore, the non-connected pads contribute to:

- increase the electric field in the fusion area
- create a topography of the electric field to induce cell trapping

In the next section we will present the device fabrication before introducing the experimental results obtained with biological cells.

2.2 Biodevice Fabrication

All the materials used for the fabrication of the device are biocompatible. The chip is fabricated on a quartz wafer pre-coated with a thin layer of Cr (15 nm Chromium to insure adhesion of Gold) and 150 nm Au as a primer for electroplating. Gold layer thickness is increased up to 5.5 µm in an electrolytic bath based on Potassium Aurocyanure $KAu[CN]_2$ (Dalmay et al., 2011) in order to enhance the electric field amplitude at the non-connected conductive pads. A photolithography step (using S1805 pohotresist) followed by a wet etching process (with KI/I_2 for Gold followed by Cr etchant MicroChemicals for Chromium) defined the electrodes (electric field generation) and the non-connected gold structures (cell positioning). The photoresist was then removed by acetone.

Finally, microfluidic channels were made of thick SU8-2025. That epoxy photoresist was spin-coated (500 rpm/100 rpm.s^{-1}/5 s then 3000 rpm/500 rpm.s^{-1}/ 30 s), soft baked (3 min at 65°C, 15 min at 95°C and 3 min at 65°C), insulated (160 mJ), post exposure baked (same 3 steps than the soft bake) and developed to form 25 µm high channels. To get a good adhesion of the photoresist, the device was hard baked during 2 hours at 175°C.

The device is packaged thanks to a simple microscope glass slide put on the top of the microchannel. A printed circuit board holder had been fabricated to ensure the electrical access to the micro-electrodes used for electric field generation (cell positioning (DEP) and electrofusion). A view of the final device electrically connected to the power supply through a PCB plate is shown in figure 4.

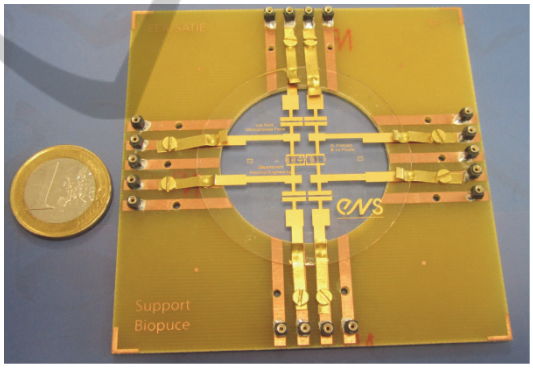

Figure 4: Global view of the fabricated chip on its PCB holder.

2.3 Biological Experiments

For biological experiments, mouse melanoma cells B16F10 had been used. They were suspended in a low conductivity hypotonic fusion buffer (0.1M Sorbitol, 0.7 mM $MgCl_2$, 0.1 mM Calcium Acetate and 1 mg/ml BSA) and injected in the fluidic channel. The diameter of B16F10 cells in this buffer is around 20 µm.

The experiments were led in static conditions. An array of 10µm*40µm gold structures separated by 50 µm has been used in order to be compatible with the size of paired cells.

2.3.1 Cell Preparation

B16F10 murine melanoma cells are cultured in Minimum Essential Medium supplemented with 10% Fetal Bovine Serum and 1% PS antibiotics (Penicilin-Streptomycin). Cultures were maintained in a 5% CO_2 incubator at 37°C. Before the experiment, cells were detached with Trypsin and suspended in the hypotonic fusion buffer (0.1M Sorbitol, 0.7 mM $MgCl_2$, 0.1 mM Calcium Acetate and 1 mg/ml BSA). The measured conductivity of the medium is 272 µS/m. Low conductivity ensures stronger pDEP and reduces Joule heating. A preparation of 1 million cells per ml was used for the experiments. The diameter of B16F10 cells in this buffer is around 20 µm.

2.3.2 Cell Trapping

With the presence of non-connected conductive pads, the application of a voltage between the electrodes produces a non-uniform electric field (figure 3). When biological cells are exposed to this field, they experience a dielectrophoretic force (Fricke, 1924):

$$\overrightarrow{F_{DEP}} = 2\pi R_{Cell}^3 \varepsilon_m \left(\Re_e [K_{CM}]\right) \vec{\nabla} \left|E^2\right| \quad (1)$$

where R_{Cell} is cell's radius, E the electric field, K_{CM} the Clausius-Mossotti (CM) factor, $\Re_e[K_{CM}]$ its real part and ε_m the extracellular medium permittivity. K_{CM} depends on the applied frequency and the electric parameters of the cell and the medium. When K_{CM} is positive, cells are attracted to maximum electric field areas, it is positive DEP (pDEP).

The DEP signal consists of a sine wave. This force is convenient to move polarisable particles. It was investigated for carbon nanotubes or other particles (Krupke et al., 2004), cell sorting and trapping (Salmanzadeh et al., 2012) or, as in our case, cell pairing.

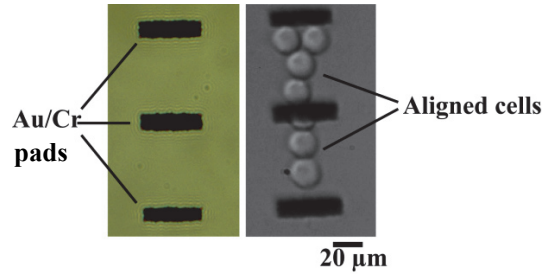

Figure 5: Top view of the non-connected gold pads arrayed within the microfluidic structure (left) and aligned cells due to positive DEP force (right).

Experiments describing cell trapping and pairing are presented in figure 5. A sine wave of 15 Vpp and 400 kHz was used during the experiment to induce DEP force.

We can see in figure 5 that cells align between the non-connected pads according to the pDEP arrows predicted by the simulation shown in figure 3.

2.3.3 Electrofusion

When the cells were paired, electrofusion pulses were added to DEP signal (10 square pulses of 100 µs duration, equivalent electric field = 1.21 kV/cm). Successful fusion had been obtained from cells in the positioning area, between two non connected gold pads (Figure 6).

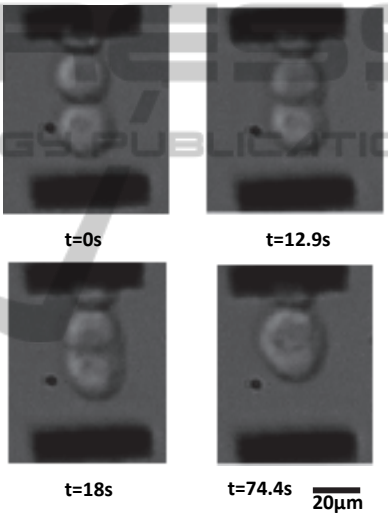

Figure 6: Dynamics of cell fusion induced by electric pulses.

After 12 seconds, the membranes of cells start to merge to form a hybridoma which regained a spherical shape 74 seconds after the first pulse.

3 CONCLUSIONS

In this paper we presented a novel structure using non-connected electroplated gold pads for high density cell pairing and fusion. The simulation of the electric field shows the effect of the conductive pads on the field distribution. Indeed, the presence of the pads increases the electric field in the fusion areas without increasing the applied voltage. On the other hand, due to the non-homogeneity of the electric field, these pads permit the use of Dielectrophoresis

to trap cells prior to electrofusion. We demonstrated the successful pairing and electrofusion between the non-connected pads with biological cells. Thanks to the absence of wiring, this method is a promising technique for high density cell electrofusion on chip.

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