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NANOCOMPOSITE CARBON-PDMS THICK ELECTRODES FOR ELECTROKINETIC MANIPULATION DURING CELL FUSION

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ABSTRACT

This paper reports on the integration and evaluation of robust, thick Carbon-PDMS nanocomposite electrodes (C-PDMS), integrated in PDMS microfluidic systems to carry out electric field induced fusion of biological cells. Such material preserves PDMS processing properties and sustains a large range of electric field intensities and frequencies without any carbon release. Polystyrene particles, yeasts and HEK-293 cells were manipulated in devices consisting of C-PDMS walls bounded to a glass plate. Different collective behaviors were observed such as alignment in chains parallel to the electric field and in circulating bands tilted with respect to the electric field direction. An AC electrofusion protocol was also tested.

KEYWORDS: Carbon-Polydimethylsiloxane Nanocomposite, Electrokinetics, Microfluidics, Cell Electrofusion

INTRODUCTION

Thick electrodes are well suited to apply uniform lateral electric fields on a cross section of the channel, which favors successful electrical cell fusion [1,3]. Beyond this purpose, such electrodes are also useful for other electrokinetic functions (cell lysis, cell trapping....). The nanocomposite carbon-PDMS (C-PDMS) conductive polymer is an advantageous material to realize such thick electrodes for electrokinetic operations in microfluidics. C-PDMS preserves PDMS processing properties that allow patterning 3D conducting microstructures, which can sustain a large range of electric potentials. In this work, we have fabricated and evaluated a microfluidic device with integrated C-PDMS electrode for cell electrofusion.

Different approaches are developed to obtain thick electrodes: UV-LIGA for metallic electrodes [2], DRIE for highly doped silicon [3] and photoresist pyrolysis for carbon [4]. Depending on the process, they can suffer from process complexity, high temperature requirement or high cost. Moreover, metallic and carbon electrodes cannot be plasma bonded with PDMS (tightness issues).

C-PDMS nanocomposite polymer combines the convenient properties of carbon (wide electrochemical window, chemically inert, biocompatible) and those related to PDMS processing (soft lithography, O₂ plasma bounding...). Electromechanical properties of C-PDMS were already exploited in the design of capacitive pressure sensors [5] or for electrochemical analysis [6]. C-PDMS was also used to control electrorheological fluid to achieve microfluidic functions [7], but to our knowledge it was never employed to manipulate cells.

EXPERIMENTAL

The nanocomposite conductive polymer was prepared by mixing carbon nanopowder and PDMS. Conductivities of 10 S.m⁻¹ were reached for a carbon concentration of 25 %. Microfluidic channels with C-PDMS walls were fabricated by soft lithography and such devices were bounded to a glass substrate by O₂ plasma activation. The photo in figure 1 represents a microfluidic device obtained following this process.

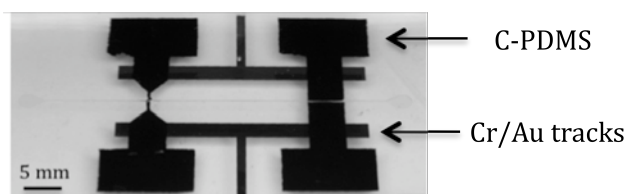


Figure 1. Photograph of the microfluidic device with a 200 μm wide channel and C-PDMS electrodes. Gold tracks providing ohmic contact are visible.

RESULTS AND DISCUSSION

First we have evaluated C-PDMS robustness for electrokinetics applications in microfluidics. Electric fields with frequencies ranging from DC to 300kHz and strengths up to $37.5 \times 10^2 \text{ V.cm}^{-1}$ were applied at the electrodes and we have checked with a particle size analyser that no carbon was released. Electrodes sustained intense DC electric potentials, up to 75 V, without being altered despite sample electrolysis. In comparison, thin metallic electrodes would have been destroyed under low frequency electric fields and metals would have been dissolved and would pollute the solution.

Our purpose in this work is to perform cell electrofusion with C-PDMS electrodes. Cell electrofusion protocol usually consists of three phases: cell alignment by dielectrophoresis (AC sinusoidal field) prior to membrane breakdown in the cells contact region (DC pulses) and membrane reconnection (AC sinusoidal field). In the context of preliminary studies, our device was validated by applying electric fields with various frequencies and strengths through a 200 μm wide

microfluidic channel containing polystyrene particles in water. AC electric fields were applied and various particle behaviors were observed depending on the field frequency. For $250 \text{ V}\cdot\text{cm}^{-1}$ electric fields, frequencies ranging from 0.5 Hz to 500 kHz were tested. For high frequencies, dipolar interaction between particles generated well-known ordered chains oriented parallel to the electric field [8]. Such an alignment is useful for the first step of cell fusion. Around 1kHz, a less-known particle organisation arose [8]. Such behavior is explained by the phase lags between three parameters: the polarization of particles, the mutually induced electric field and the applied electric field, generating the spinning of particles. These tangential hydrodynamic forces induced by the spinning can override the dipole-dipole attraction at those frequencies. In our configuration, we observed that particles were spinning and forming bands tilted with angles of around 60° - 45° with respect to the electric field direction. In the very low frequency range (a few Hz), electric fields induced electrophoretic motion of particles. All these behaviors demonstrate the wide application range of these C-DPMS electrodes and are illustrated in figure 2.

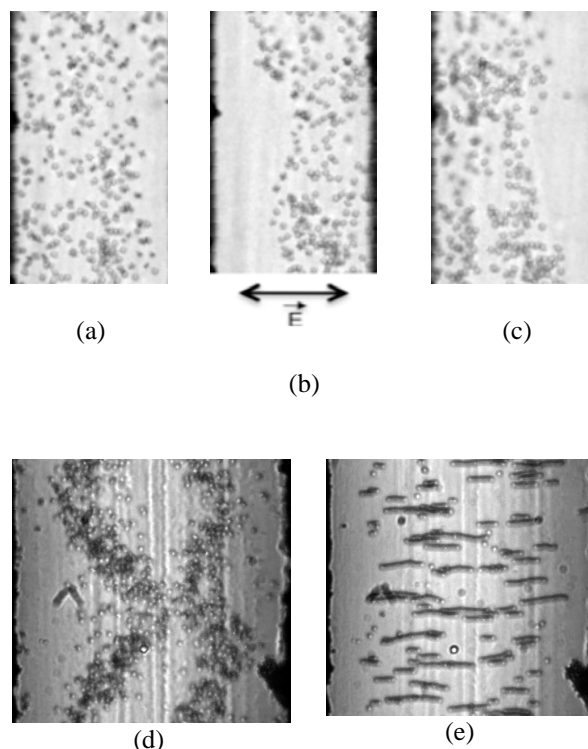


Figure 2: Particle (polystyrene $10\mu\text{m}$ diameter (a,b,c) and $3\mu\text{m}$ diameter (d,e)) behaviors under $250\text{V}/\text{cm}$ electric fields at different frequencies: (a) no applied electric field, no organization is noticed, (b,c) 1Hz, particles move from one electrode to the other under electrophoretic forces, (d) 1kHz, particles gather in circulating bands, tilted with respect to the electric field direction and (e) 10kHz, particles form chains oriented along the applied field direction.

Then, we demonstrated manipulation of biological cells such as yeasts and HEK-293 cells (Human Embryonic Kidney cell line, ATCC, CRL-1573). Figure 3 shows yeasts aligned parallel to the high frequency electric field direction.

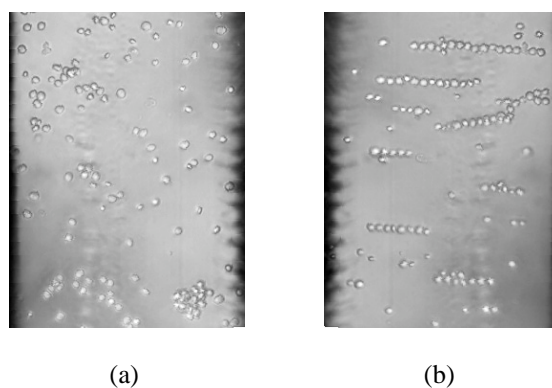


Figure 3: (a) Yeasts without applied electric field; (b) Yeasts forming chains aligned parallel to the applied electric field ($500 \text{ V}/\text{cm}$ 500 kHz)

An electrofusion protocol involving high strength electric pulses was tested on HEK293 cells. Prior to the experiment,

cells were trypsinated, washed and resuspended in a low-conductive PSB (0.1 M) medium. The pH and conductivity of this solution were respectively adjusted to 7.4 and $100 \mu\text{S}\cdot\text{cm}^{-1}$. This solution was supplemented with saccharose to set the osmolarity to 300 mOsm. During the application of the AC sinusoidal field, cells gathered in close contact. Figure 4 presents cells in contact before and after DC pulses.

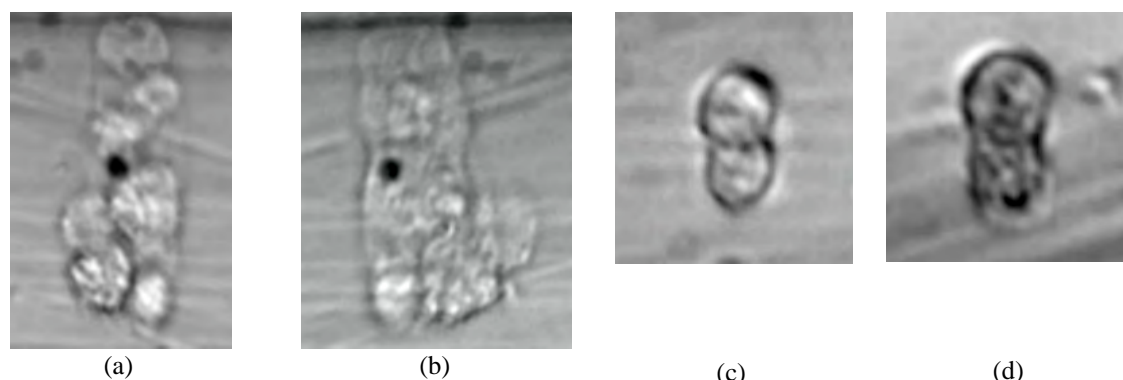


Figure 4: Electroporation protocol on HEK-293 cell cluster (a, b) and a pair of cells (c, d): under AC sinusoidal fields (375 V/cm , 10 kHz) cells are in close contact (a, c), after DC pulses (3750 V/cm) of $100 \mu\text{s}$ every 1 ms (b, d).

CONCLUSION

C-PDMS proves to be a robust conductive material that can sustain a large range of electric fields without carbon release. It can be patterned in thick or 3D electrodes, which answers a need for applications requiring uniform electric field in the entire channel. With the view of performing cell electroporation, we have used C-PDMS electrodes to manipulate polystyrene particles and observed different behaviors depending on the applied electric field. We have then electrically aligned yeasts parallel to the electric field, which is the first step of the electrical fusion protocol and experimented electroporation on HEK293 cells.

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