



nDEP-driven cell patterning and bottom-up construction of cell aggregates using a new bioelectronic chip



S. Menad^a, L. Franqueville^a, N. Haddour^{a,*}, F. Buret^a, M. Frenea-Robin^{b,*}

^a Université de Lyon, Ecole centrale de Lyon, CNRS UMR 5005, Laboratoire Ampère, 69130 Ecully, France

^b Université de Lyon, Université Lyon 1, CNRS UMR 5005, Laboratoire Ampère, Villeurbanne F-69622, France

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ABSTRACT

Creating cell aggregates of controlled size and shape and patterning cells on substrates using a bottom-up approach constitutes important challenges for tissue-engineering applications and studies of cell–cell interactions. In this paper, we report nDEP (negative dielectrophoresis) driven assembly of cells as compact aggregates or onto defined areas using a new bioelectronic chip. This chip is composed of a quadri-polar electrode array obtained using coplanar electrodes partially covered with a thin, micropatterned PDMS membrane. This thin PDMS layer was coated with poly-L-lysine and played the role of adhesive substrate for cell patterning. For the formation of detachable cell aggregates, the PDMS was not pre-treated and cells were simply immobilized into assemblies maintained by cell–cell adhesion after the electric field removal. Cell viability after exposition to DEP buffer was also assessed, as well as cell spreading activity following DEP-driven assembly.

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1. Introduction

Developing new devices to pattern cells into regular networks is a major concern in the field of bioelectronics research, tissue engineering [1] and biotechnology. Cell immobilization in defined areas allows the study of cell–cell interactions, cell morphology and differentiation. Spatial patterning of cells in microfluidic systems is an essential step in the development of cell-culture platforms for drug testing that reproduce *in vivo* cellular microenvironment [2,3].

Among the approaches currently investigated to engineer biomaterials that mimic the structure of native tissues, one promising route is the development of bottom-up cell assembly [4], which consists in fabricating tissues from small modules consisting of cells brought together by self-assembly or directed assembly. Compared to prevalent top-down molding approaches, these modular tissue-engineering techniques provide better control over the relative spatial organization of cells [5].

One solution to direct and assemble cells into desired areas consists in using magnetic, optical or electrokinetic forces. Such approaches share the common advantage of accelerating the establishment of cell–cell interactions. It is of great interest, as aggregation can play a significant role in a number of mechanisms controlling cell physiological state [6]. Magnetic fields can be used

to perform scalable cell assemblies in various types of solutions, including cell culture medium. Three-dimensional tissue culture can be achieved based on magnetic levitation of cells and cell culture shape can be engineered by spatially controlling the magnetic field [7]. However, this method involves the uptake of magnetic nanoparticles by cells, which might be undesirable. Optical tweezers technology [8] offers very high resolution for the study and replication of micro-assemblies, but is not adapted to large scale massive in-parallel cell manipulation due to tight focusing requirements [9].

On the other hand, the dielectrophoretic force permits to handle cells in a wide size range using label-free protocols and allows the rapid construction of regular cell patterns with high resolution [10]. This force arises when a polarizable particle is subjected to a non-uniform electric field. DEP can be used to direct cells toward high or low electric field areas, phenomena referred to as positive (pDEP) and negative (nDEP) dielectrophoresis, respectively.

DEP enables the creation of aggregates of various sizes, shapes and spacings, from different cell types and to pattern large number of cells in parallel. In addition to numerous applications involving eukaryotic cell patterning, DEP has been used for bacterial cell organization in microcolonies [11]. Within these DEP-constructed microcellular architectures, the study of cellular interactions was successfully investigated [12,6].

To preserve the specific microorganization of cells, the structures obtained by force-guided cell assembly can be stabilized by crosslinking the cells using a flocculant [11] or trapping them in

* Corresponding authors at: 36 Avenue Guy de Collongue, 69130 Écully, France. Tel.: +33 4 72 18 61 12 (M. Frénea-Robin), +33 4 72 18 61 12 (N. Haddour).

E-mail addresses: naoufel-haddour@ec-lyon.fr (N. Haddour), marie.robin@univ-lyon1.fr (M. Frenea-Robin).

a biocompatible hydrogel in which they remain viable for several days [13–17]. However, cell embedding in hydrogel is irreversible, which may be a drawback in some applications. DEP cell manipulation can also be combined with surface functionalization to drag cells toward chemically treated adhesion areas, where they remain trapped after the electric field is turned off. Once cells have adhered on the surface, the low conductivity buffer usually employed in DEP experiments can be easily replaced with cell growth medium, thus minimizing the detrimental effects on cells. Indeed, pDEP was used to trap cells on electrodes and enable their immobilization in high electric field regions, selectively coated with fibronectin [18]. Ho and coworkers also reported rapid heterogeneous liver-cell on-chip patterning using pDEP. In this work, cell adhesion onto the surface was enhanced by supplementing the medium with Ca^{2+} and Mg^{2+} and coating the glass substrate with poly-D-lysine [10]. In a more recent study, the same authors used Type I collagen as extracellular matrix (ECM) and cell-adhesion promoter coated on the biochip substrate [19]. Adherent cells could also be immobilized in aggregates formed by pDEP thanks to electric-field inducing cell–cell contact, if the electric field was maintained for a sufficient period of time [20]. However, cell patterning can also be achieved using nDEP [15,21,22]. This brings the advantage of minimizing cell damage, as the force pushes them toward regions of lower electric field intensity [23]. Moreover, cells can be tightly focused in compact aggregates by nDEP, using specific electrode designs. In particular, quadrupolar electrode structures allow cell concentration toward isolated, well-defined, electric field minima [24,25].

We have recently reported a simple and low-cost process for fabricating quadrupolar electrode arrays using bond-detach lithography [26]. This fully transparent microchip is obtained by performing selective openings in a thin PDMS membrane deposited onto a bipolar interdigitated electrode structure made of indium thin oxide (ITO). In this paper, we show that this device allows nDEP-driven formation of living cell aggregates without addition of immobilization agents. Moreover, the materialized cell aggregates might be collected after the electric field is turned-off and further cultivated in regular cell growth medium. We also demonstrate successful cell patterning on the microchip surface, by coupling DEP with poly-L-lysine treatment of the PDMS membrane.

2. Theory

Dielectrophoresis is defined as the motion of polarizable particles subjected to non-uniform electric fields. The dielectrophoretic force acting on a particle scales with the gradient of the squared electric field intensity, as shown by Eq. (1) [27,28]

$$F_{\text{DEP}} = 2\pi\epsilon_m r^3 \text{Re}[\text{CM}] \nabla |E|^2 \quad (1)$$

where ϵ_m is the permittivity of the immersion medium and r is the particle radius. E refers to the rms strength of the electric field and $\text{Re}[\text{CM}]$ is the real part of the Clausius–Mossotti (CM) factor, which can be further expressed as:

$$\text{Re}(\text{CM}) = \text{Re}\left(\frac{\tilde{\epsilon}_p - \tilde{\epsilon}_m}{\tilde{\epsilon}_p + 2\tilde{\epsilon}_m}\right) \quad (2)$$

$$\tilde{\epsilon} = \epsilon - j\frac{\sigma}{\omega} \quad (3)$$

$\tilde{\epsilon}_p$ and $\tilde{\epsilon}_m$ represent the complex permittivities of the particle and its immersion medium, which depend on their respective dielectric properties (conductivities σ_p , σ_m and permittivities ϵ_p , ϵ_m) and on the electric field angular frequency ω . By using the so-called single-shell dielectric model [29], the effective complex cell permittivity of a biological cell can be written as:

$$\tilde{\epsilon}_p = \frac{\left(C_{\text{memb}} + \frac{G_{\text{memb}}}{j\omega}\right)r \times (\epsilon_0\epsilon_{rc} + \frac{\sigma_c}{j\omega})}{\left(C_{\text{memb}} + \frac{G_{\text{memb}}}{j\omega}\right)r + (\epsilon_0\epsilon_{rc} + \frac{\sigma_c}{j\omega})} \quad (4)$$

where σ_c and ϵ_{rc} represent the conductivity and relative permittivity of the cytoplasm. G_{memb} and C_{memb} refer to cell area-specific membrane conductance and capacitance, respectively.

Using Eqs. (1)–(4), the dielectrophoretic behavior of cells can therefore be predicted from the knowledge of their dielectric properties (C_{memb} , G_{memb} , σ_c , ϵ_{rc}) and radius r .

According to the force expression, when cells are more polarizable than the surrounding medium ($\text{Re}(\text{CM}) > 0$), the DEP force acts up the field gradient toward the region of highest electric field intensities (pDEP). The opposite case ($\text{Re}(\text{CM}) < 0$) corresponds to nDEP, where cells are repelled from these regions.

3. Materials and methods

3.1. Microchip fabrication

High-density quadrupolar electrode arrays were fabricated using a recently developed technique adapted from bond-detach lithography [30] and following protocols described elsewhere [23]. Briefly, the process relies on the transition from a bipolar to a quadrupolar electrode arrangement, which is achieved by partially covering the bipolar electrode surface with a thin micropatterned PDMS membrane acting as an electrical insulation layer. Three major steps are involved in this process, as summarized in Fig. 1. The first one consists in fabricating an interdigitated electrode array made from Indium Tin Oxide on glass. Each electrode branch consists of $150\ \mu\text{m} \times 150\ \mu\text{m}$ square patterns disposed in staggered rows, two adjacent squares being separated by a $250\ \mu\text{m}$ long and $30\ \mu\text{m}$ wide track (Fig. 1a). The surface of this electrode array is then coated with a thin (200 nm-thick) PDMS layer (Fig. 1b). Finally, a PDMS stamp is pressed against the substrate, leaving selective openings in the PDMS insulating membrane after retrieval, thus producing an array of quadrupolar electrode sets (Fig. 1d).

3.2. Bioelectronic chip surface functionalization using poly-L-lysine

Poly-L-lysine surface treatments were performed to enhance cell adhesion on patterned PDMS layer. $200\ \mu\text{l}$ of a 0.01% poly-L-lysine sterile solution, 70000–150 000 mol wt, (Sigma, France) were aseptically added on a patterned PDMS layer and incubated at room temperature for 10 min. The excess of the solution was removed by rinsing with deionized water and then two times with Phosphate Buffered Saline (PBS) solution. The bio-chip was air-dried at room temperature prior to cell-patterning experiments.

3.3. Cells and media

Human embryonic kidney 293 (HEK-293) cells were obtained from CelluloNet, UMS3444/US8 BioSciences Gerland Lyon-sud (Lyon, France) and maintained at $37\ ^\circ\text{C}$, under 5% CO_2 in Dulbecco's Modified Eagle medium–High glucose supplemented with 10% fetal calf serum, 1% non essential amino acids (100 \times), and 0.1% Sodium Pyruvate (10 mM) (PAA, France).

Prior to dielectrophoresis experiments, sub-confluent HEK-293 cell monolayers were harvested by trypsin/EDTA (1 \times) treatment. The trypsin was neutralized by adding cell culture medium. HEK-293 cells were pelleted by centrifugation at $230\times g$ for 5 min and resuspended in 3 ml of low conductivity, isotonic medium. This DEP buffer was prepared by mixing deionized water with PBS until obtaining a conductivity of 50 mS/m (measured with a Consort C532 conductimeter). Dextrose was then dissolved in this buffer

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