Topic: Biomedical and Chemical Micro Sensors and Systems

A NEW MEA FOR SINGLE-SITE MULTIPLE TRANSFECTIONS: SURFACE FUNCTIONALIZATION AND MICROFLUIDICS INTEGRATION

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Nowadays, different chemical and physical transfection techniques are used to delivery biomolecules of interest (e.g. DNA, RNA, proteins) into cells. Among the physical methods, electroporation generates transient pores in the plasma membrane by applying electrical pulses to suspended cells. One of its main limitations is the lack of spatio-temporal control over the process: it does not allow to select single cells (desirable requirement especially in highly heterogeneous tissues), and to monitor the transfection results in real-time. To circumvent these disadvantages, alternative microscale approaches are increasingly required [1].

This work presents an integrated platform consisting of two modules (Fig.1): a gold microelectrode array (MEA) for single-site electroporation and two interdigitated electrode arrays (IDEs) for the dielectrophoretic (DEP) and controlled delivery of bio-chemical entities (Fig.2). Both modules are characterized by two levels of metal structures (buried connection lines made of Al 1% Si + Ti/TiN and Gold electrodes) in order to reduce the fabrication costs and the dimensions while improving the device electrical performances. Biocompatible quartz is used for cells confinement and independent microfluidic channels, obtained by using anisotropic wet etching, permit to inject various bio-chemical species into different cell groups through a 4 µm hole dug at the centre of each chamber (single-site delivery). Furthermore, nanostructured TiO_2 is deposited by means of Pulsed Microplasma Cluster Source (PMCS) in order to pattern the electrode active areas and improve cell biocompatibility. The modules have been realized and separately tested in order to verify the feasibility of the approach. The biocompatibility of the materials employed in the microfabrication has been yet demonstrated and reported elsewhere [2]. Single-site electroporation has been performed by applying a voltage directly on adherent cells, while the transfection solution was injected into a specific area of the chip through a microfluidic channel (Fig. 3). Moreover, the dielectrophoretic effects of the device have been evaluated, as case study, by using two types of microsized polystyrene beads. Controlled movement and also separation of these different size particles has been obtained applying alternating electric field over the electrodes at specific operative frequency and voltage (Fig. 4). Both DEP and a pressure-driven flow are considered in order to optimize the delivery system.

Thanks to its structure, which can permit multiple *in-vitro* assays, this versatile integrated platform may provide an useful tool for high-throughput single-site analysis in drug discovery and basic biomedical research.

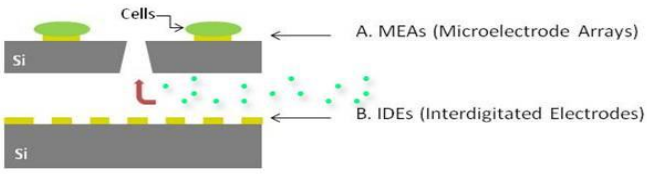

Figure 1. Schematic illustration of the integrated system: **(A)** MEAs for cell electroporation and **(B)** IDEs for addressed cell drug delivery.

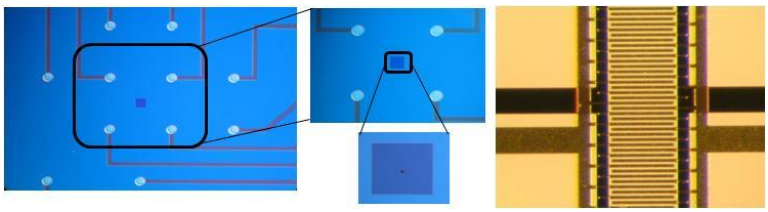

Figure 2. **(Left)** MEA for single-site electroporation and passing hole for transfectants' delivery**. (Right)** IDEs structure: electrode width and gap of 10 µm.

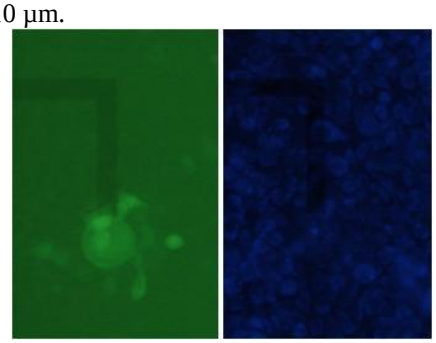

Figure 3. Fluorescence micrographs of HeLa cells electroporation with Lucifer Yellow (0.5 mM): **(Left)** specific uptake of the fluorescent dye and **(Right)** autofluorescence view of the whole cell population.

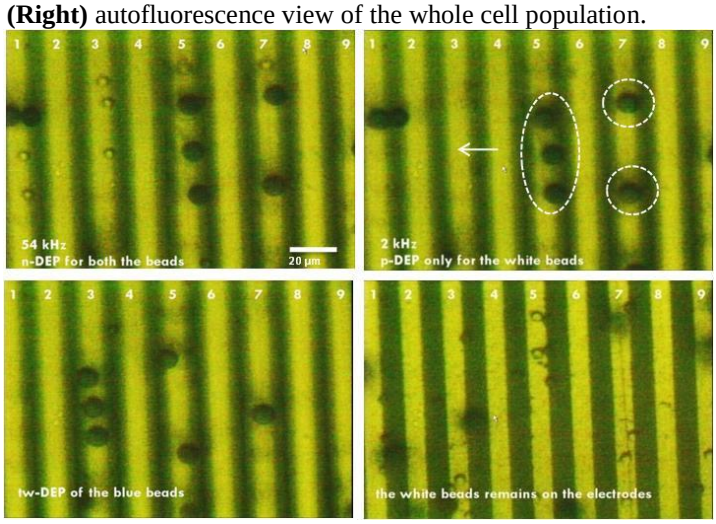

Figure 4. The picture shows (clockwise direction) four phases of the separation of different size polystyrene microparticles (white 5 µm beads and blue 10 µm beads): negative DEP (n-DEP) for both beads (54 kHz), positive DEP (p-DEP) for the white beads and n-DEP for the blue beads (2 kHz). At the same frequency the blue beads laterally move (travelling wave DEP, tw-DEP), while the white beads are retained on the electrodes (p-DEP).

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