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An enhanced platform for cell electroporation: controlled delivery and electrodes functionalization

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Abstract

This work presents an improved platform for single-site electroporation and controlled transfectants' delivery. The device consists of a gold microelectrode array (MEA) with integrated microfluidics and nanostructured titanium dioxide (ns-TiO_2) functionalized electrodes. Human cervical cancer cells (HeLa) have been successfully cultivated on chip surface. The system has been previously tested by electroporating them with Lucifer Yellow (LY) and then, in order to validate the approach and cell viability, with plasmids for the enhanced expression of Green Fluorescence Protein (pEGFP) delivered through microchannels.

Keywords: Microelectrode Array (MEA); Cell Electroporation; Microfluidics; Functionalization.

1. Introduction

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 [1] 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. This work presents the improvements of an electroporation system [2] comprising multiple cell compartments, underlying microfluidics, and patterning of the electrode active areas with ns-TiO_2.

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2. Materials and methods

2.1 Device microfabrication

The electroporation module consists of a gold cell-size microelectrode array (MEA) and it is 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. Passing holes allow the delivery of transfection solutions from the microfluidic structure (integrated below the MEA) into desired areas of the chip. In detail, the microfabrication process starts from a 500 µm thick silicon wafer (double side polished). A multilayer of dielectrics is grown and deposited on the top of silicon ($SiO_2/Si_3N_4/SiO_2$ ~1µ of thickness), in order to insulate the electrodes and realize the membrane for the injection system. An Al 1% Si + Ti (410/60nm)/ TiN (140nm) multilayer is deposited and patterned by photolithography and plasma dry etching to form electrodes, lines and contact pin zones. A layer of Si_3N_4/SiO_2 (220nm) is then deposited by plasma enhanced chemical vapor deposition (PECVD) for insulating the metal lines of the electrodes. Contacts are opened through the Si_3N_4/SiO_2 layer by a plasma dry etching. A layer of 5 nm of chromium and 150 nm of gold are evaporated and patterned using wet etching. A bulk wet etching is performed on the wafer back side to define the areas for microchannels and sample delivering. Finally a dry etch plasma is carried out in order to complete the injection area for the solution. A thick structured layer of PDMS polymer with the silica pipes is bonded on the bottom of the chip in order to complete the fluidic system. A three dimensional cell confinement structure is realized by using isotropically etched 500 µm-quartz wafer. The glass wafer is masked with 500 nm of undoped poly-silicon and etched with HF 40% at an etching rate of 37.5 µm/hour. Finally it is glued on the chip with epoxy resin.

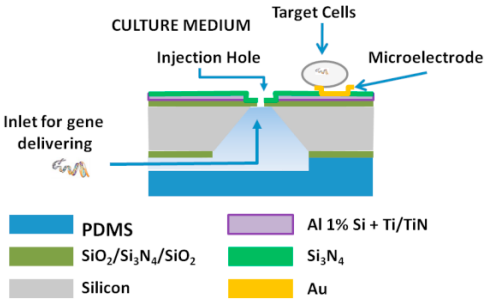

Figure 1. Schematic cross section of the integrated system and fabrication process outline.

2.2 Electrode nt-TiO_2 functionalization and final packaging

After the fabrication of the microelectrode array, ns-TiO_2 is deposited by means of a Pulsed Microplasma Cluster Source (PMCS) [3] in order to improve cell biocompatibility and adhesion. The morphology of ns-TiO_2 film is characterized nano grains ranging from 8 to 20 nm in size, with the presence of distributed larger cluster in the micron scale and with nano crystalline structure both in Anatase and Rutile phase. In particular, a fabrication step based on lithography and lift-off is implemented in order to allow the functionalization of the gold electrodes, specifically in the active zone (Figure 2), where the voltage is applied to adherent cells. The whole device is covered with a photoresist patterned in such a way to leave the active areas of the electrodes exposed to the beam. After the PMCS nt-TiO_2 deposition the photoresist is removed together with the unwanted TiO_2.

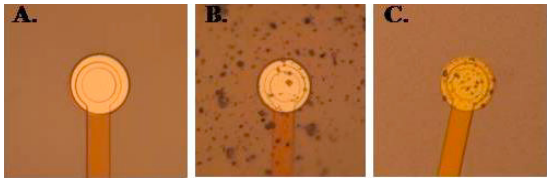

Figure 2. Nanostructured TiO_2 functionalization process: (A) microelectrode for electroporation before deposition; (B) TiO_2 deposition by means of PMCS on the whole electroporation array, after photoresist patterning; (C) photoresist removal together with the unwanted TiO_2.

In Figure 2, the TiO_2 deposition is visible only on the electrodes, while the remaining regions are clean. The presence of microscopic droplets is due to characteristic process of the cluster formation, where local zones of higher temperature allow the aggregation of larger clusters.
Finally, the chip is inserted in a custom PCB and in a glass cell confinement structure. Moreover, fused silica tubes are added to complete the fluidic connections, as reported in Figure 3.

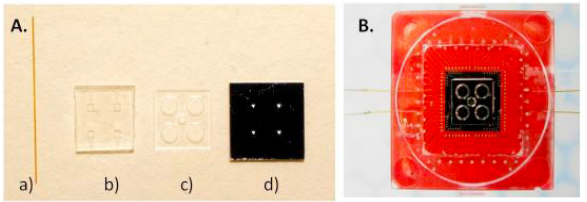

Figure 3. (A) Details of the chip components: a.) fused silica tube; b) PDMS structure for microfluidic injection; c) quartz structure for cell confinement; d) back of silicon chip with injection holes; (B) picture of the packaged device.

2.3 Experimental method

After a deep cleaning and UV treatment (sterilization) of the devices, cervical cancer HeLa cells are successfully cultivated using traditional protocols. 24 hours later, single-site electroporation is performed by applying an electric pulse directly on adherent cells and by injecting the transfectant solution through microchannels.
The measurement setup consists of a signal generator, a microscope and a PCB board in which each chip is mounted. Different parameters are investigated: the voltage amplitude, the pulse duration, the cell and transfectant concentrations.
In order to evaluate the feasibility of the technique a small (475 Da) fluorescent dye (Lucifer Yellow, LY) has been employed. After 5 minutes incubation (light protected) and washing with a saline buffer, the electroporation result has been observed under fluorescence microscopy. In order to assess the possibility to perform gene expression and to evaluate the cell viability after electroporation, other tests have been performed by transfecting plasmids for the enhanced expression of Green Fluorescence Protein (pEGFP). The uptake and the successful gene expression have been evaluated after 10 and 24 hours.

3. Experimental results: single-site electroporation

Device electrical characterization - electrochemical impedance spectroscopy (EIS) and voltammetry - and biocompatibility study of the materials employed in microfabrication process (demonstrating the improvement of cell adhesion in presence on TiO_2 functionalization) have been already performed and reported elsewhere [4, 5]. Successful single-site electroporation has been obtained by applying an electric pulse (6 V, 100 μs) directly on HeLa cells. Figures 4A and 4B demonstrate the specificity of the transfection: only the four cells positioned on the selected electrode have been specifically stained, thus demonstrating, in that region, the permeabilization of the membrane due to the opening of transient pores.

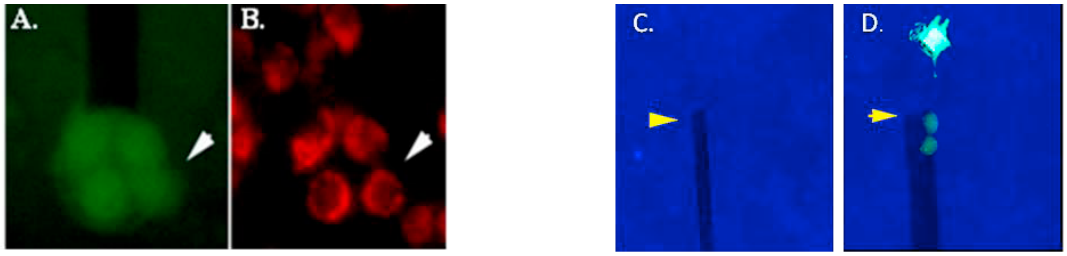

Figure 4. (*Left*) Fluorescence micrographs of HeLa cells electroporation with Lucifer Yellow (0.5 mM): (A) specific uptake of the fluorescent dye; (B) autofluorescence view of the cell population (20X). (*Right*) Fluorescence micrographs of HeLa cells electroporation with pEGFP (0.5 µg/µl): (C) autofluorescence view of the single cell; (D) specific uptake of the plasmids with consequent expression of GFP (20X).

An higher electric pulse (7 V, 100 µs) has been applied in order to delivery pEGFP into cells (Figures 4 C and 4 D). After 10 and 24 hours from electroporation the cell was able to successfully express the fluorescent protein.

4. Conclusions

The evolution from the original configuration (i.e. quince matrix of 60 microelectrodes) with microfluidic channels and functionalized electrodes has satisfied the need to have various microenvironments for testing different electroporation solutions with improved bioaffinity and good electroporation efficiency. In particular, independent microfluidic channels have permitted to inject various biochemical species into different cell groups through a 4 µm hole dug at the centre of each chamber (single-site delivery). Moreover, the functionalization of the active area of the chip has improved bioaffinity and especially cell adhesion. Both electroporation and cell vitality after 24 hours from tests have been demonstrated by using a small fluorescent dye and performing gene expression.
Thanks to its structure, which can allow 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.

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