FUNCTIONALIZED MICROELECTRODES ARRAYS WITH INTEGRATED MICROFLUIDIC CHANNELS FOR SINGLE-SITE MULTIPLE TRANSFECTIONS

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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 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 a gold microelectrode array (MEA) for single-site electroporation and fluidic channels for controlled delivery of bio-chemical entities (Fig.1). In order to improve the efficiency of electroporation, the gold electrodes were coated with a thin film of nanostructured of Titanium Dioxide [2-3].

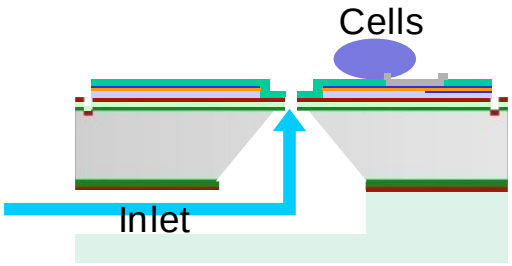

Figure 1. Schematic cross section of the integrated system consisting of a MEA for cell electroporation and microfluidic channels for transfectants' delivery**.**

MATERIAL AND METHODS

MEAs devices were fabricated using 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). After the fabrication of the array, a thin film of nanostructured TiO_2 was deposited only on the electrode active areas by means of a Pulsed Microplasma Cluster Source (PMCS) in combination with a lift-off step: the whole device was covered with a photoresist patterned in such a way to leave the electrodes exposed to the beam. After the deposition, the photoresist was removed together with the unwanted TiO_2. After a deep cleaning and UV treatment (sterilization) of the devices, human cervical cancer cells (HeLa) were successfully cultivated using traditional protocols. 24 hours later, the electroporation experiments started by applying an electric pulse (6/7 volts, 100µs) directly on adherent cells, while the transfection solution (plasmids for the gene expression of the green fluorescent protein – pEGFP - 0.5 µg/µl) was injected into a specific area of the chip through the microfluidic channel (Fig. 1).

RESULTS AND DISCUSSION

Figure 2 shows the results of the functionalization of the gold electrodes with TiO_2. In Figure 2(a),an electrode before the deposition is reported, while in Figure 2(c) the same electrode is showed after the functionalization with nanostructured TiO_2 deposited by PMCS technique. Figure 2(d) is a photograph of the device after lift-off. The TiO_2 deposition is visible only on the electrodes, while the remaining regions are un-functionalized. 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. A micro Raman analysis was performed on the larger clusters revealing a crystalline structure of the TiO_2 mostly in the Anatase phase.

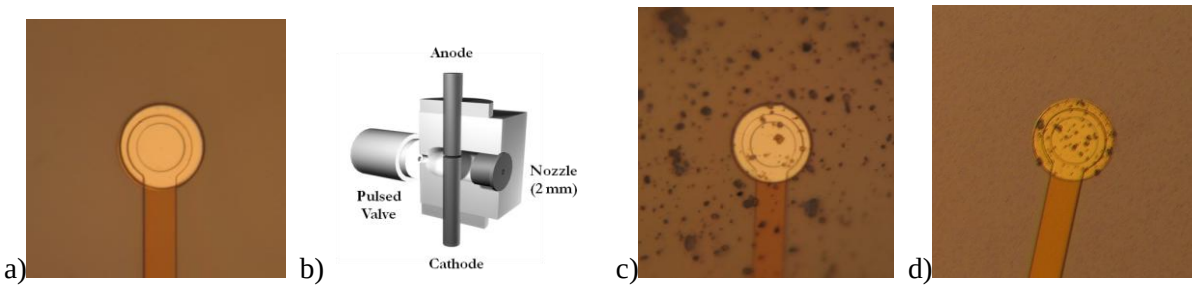

Figure 2. Microelectrode for electroporation before (a) and after (c) TiO_2 deposition with PMCS (b). After the removal of the photoresist and the unwanted TiO_2, the functionalization remains only on the active area of the electrodes (d).

Single-site electroporation was assessed after 10 and 24 hours from the application of the voltage, thus demonstrating both electroporation and cell viability.

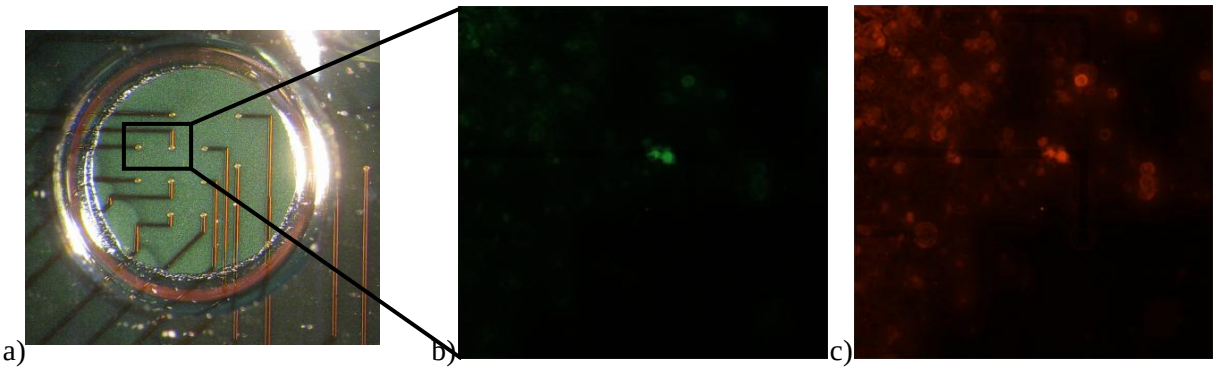

Figure 3. Fluorescence micrographs of HeLa cells electroporation with pEGFP (0.5 µg/µl): (a) Picture of the chamber containing the electrodes; (b) specific uptake of the fluorescent dye and (c) autofluorescence view of the whole cell population.

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REFERENCES

[1]. S. Vassanelli, L. Bandiera, M. Borgo, G. Cellere, L. Santoni, C. Bersani, M. Salomon, M. Zaccolo, L. Lorenzelli, S. Girardi et al. Space and time resolved gene expression experiments on cultured mammalian cells by a single-cell electroporation microarray. In *New Biotechnology* (2008), vol 25 No. 1: 55—67.

[2] N. Coppedè, T. Toccoli, F. Siviero, A. Pallaoro, S. Iannotta, A. Forleo, P. Siciliano, M. Lattore, D. Marzorati, Novel nano-hybrid sensor based on nanocrystalline TiO2 functionalized by phthalocyanines via supersonic beam codeposition: performance and application to automotive air quality, Associazione Italiana Sensori e Microsistemi (AISEM) XIII CONFERENZA ANNUALE, Roma 19-21 Febbraio 2008.

[3] C. Ress, L. Odorizzi, C. Collini, L. Lorenzelli, S. Forti, C. Pederzolli, L. Lunelli, L. Vanzetti, N. Coppede', T. Toccoli, G. Tarabella, S. Iannotta - Comparative bioaffinity studies for *in-vitro* cell assays on MEMS-based devices, *Sensors and Microsystems: AISEM 2009 Proceedings*, Lecture Notes in Electrical Engineering 54: 83-87– Springer.