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Fabrication and characterization of a fully integrated microdevice for *in-vitro* single cell assays

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Overview

The aim of this work is the development of a microdevice able to provide *in-vitro* assays at single cell level. Two modules, integrated in a single platform, are presented: interdigitated electrode arrays (IDEs)-based microsystem for the cell addressed delivery of bio-functionalized nano/microparticles and a cell size microelectrode array (MEA) for single cell electroporation. Both the 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. Additional steps of bulk micromachining are developed in order to realize the inlet microfluidics of the MEA-based module. Biocompatible polymers and quartz are used for microchannels and cells confinement respectively. In order to demonstrate the feasibility of this approach, both modules are individually characterized. The dielectrophoretic (DEP) capability of the former is demonstrated by using polystyrene microbeads and the bioaffinity of the latter is evaluated by successful Chinese Hamster Ovary (CHO) cells culture on chip. Moreover, preliminary results of electrochemical impedance spectroscopy [100Hz–1MHz] and of a Randles-based electrical model show the stability of electrode/solution interface parameters ($|Z(f)|$ dispersion < 3%) before and after the cell culture.

1. Schematic representation of the main idea

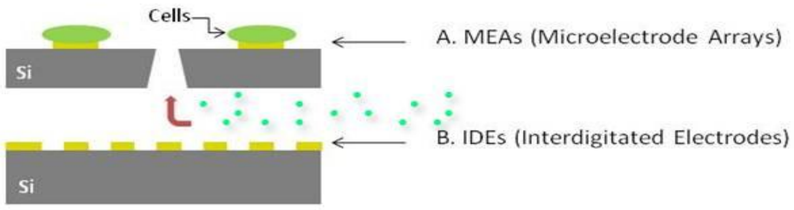


***Figure 1**. Schematic illustration of the integrated system: MEAs for cell electroporation (A) and IDEs for addressed cell drug delivery (B).*

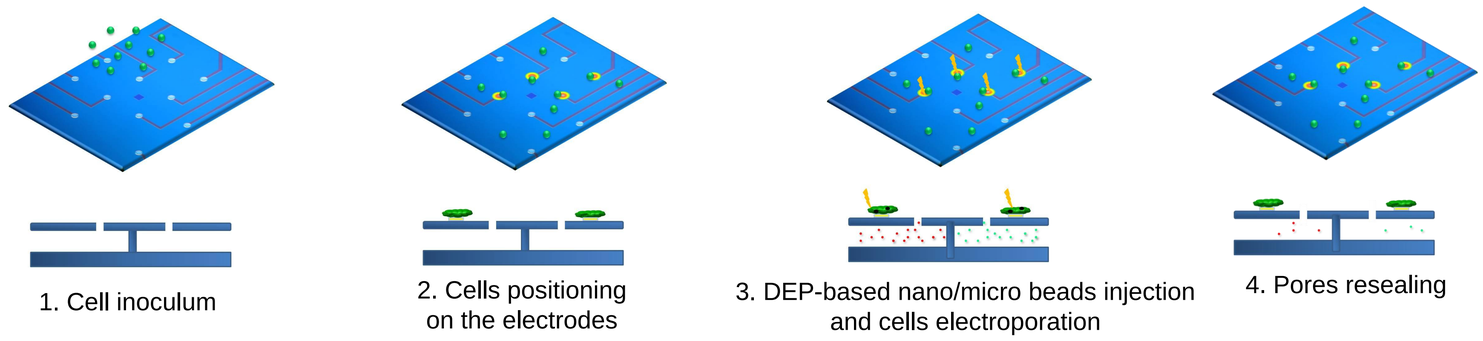


***Figure 2**. Device concept: all steps of the experimental procedure are shown.*

2A. Microelectrode array (MEA): device design

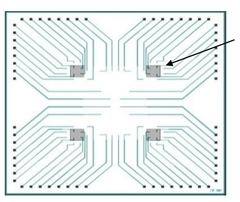


***Figure 3.** Chip layout.*

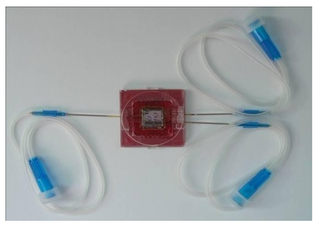


***Figure 5.** Device packaging and fluidic connections.*

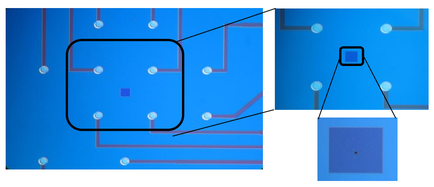


***Figure 4.** Membrane of dielectric multilayer and passing hole.*

Electrode diameter	50µm
Chip dimension	1cm x 1 cm
Membrane dimension	20-60 µm
Holes dimension	3-4 µm

***Table 1.** Geometrical parameters of the MEA-based module.*

2. Microfabrication process

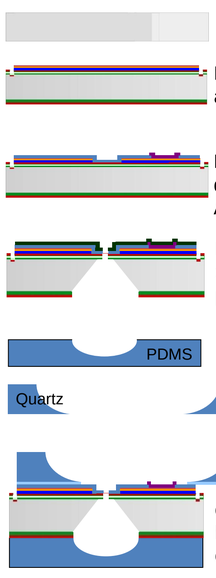


***Figure 6.** Main steps of the device microfabrication.*

2B. Interdigitated electrode arrays (IDEs): device design

The device layout has been investigated and realized in order to perform alternate electric fields with waveforms shifted by 180 and 90 respectively.

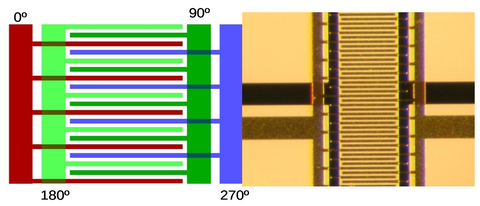


Figure 7. IDEs structure (left) and picture (right): electrode width and gap of 10 µm.

Two electric contacts: positive and negative dielectrophoresis (p-n DEP)

two waveforms with a phase shift of 180°

steady-state wave

no spatial phase variation
levitation force

Four electric contacts: travelling wave dielectrophoresis (tw DEP)

four waveforms with a phase shift of 90°

no steady-state wave
travelling wave

spatial phase variation
horizontal conveyance

3A. Bioaffinity test and device electrical characterization

Chinese Hamster Ovary (CHO) cell line has been used to check the biocompatibility of the employed materials.

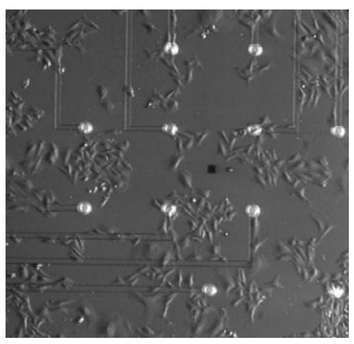
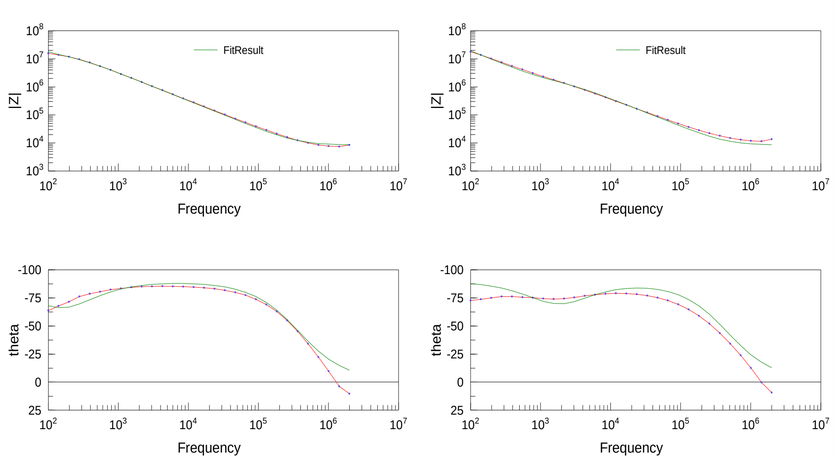


***Figure 8.** Adherent CHO cells cultivated on a MEA-based device, throughout standard procedure (2000 cells/cm^2 , 3 days incubation @ 37°C, 5% CO_2)*



Transfer resistance: 24 MΩ
Double layer capacitance: 50 pF
Diffusion capacitance: 65 pF

Transfer resistance: 3 MΩ
Double layer capacitance: 40 pF
Diffusion capacitance: 43 pF

***Figure 9.** Impedance IZI (Ohm) and Phase (Degree) vs Frequency (Hz) for electrode without (left) and with cells (right) respectively: data and fits are shown.*

Electrochemical impedance spectroscopy has been carried out to analyse the intrinsic electrical parameters of the solid device and both metal/solution-metal/cell interfaces. Lumped model (based on Randles' model) has been used to fit the data.

3B. Device testing: polystyrene beads dielectrophoretic experiments

Polystyrene microbeads with different diameters (5 and 10 µm) have been used in order to simulate two different transfectants. Their dielectrophoretic movement (both vertical and orizontal) has been performed individually and also by using a mixture of different beads.

$3V_{p-p}$ KCl solution conductivity 63µS/cm		
beads	5µm	10µm
n-DEP	54 kHz	54 kHz
p-DEP	2 kHz	0.8 kHz

***Table 2.** Frequency values for the motion of two types of beads.*

Bead VERTICAL conveyance

n-DEP
towards the minima of the electric field with levitation

p-DEP
towards the maxima of the electric field at the edge of the electrodes

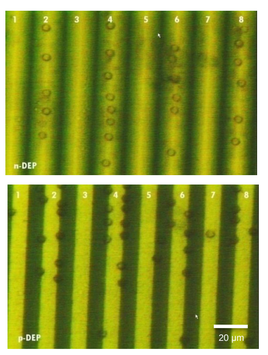


***Figure 10 .** Vertical motion (p- and n-DEP) of 5µm beads.*

Bead LATERAL conveyance

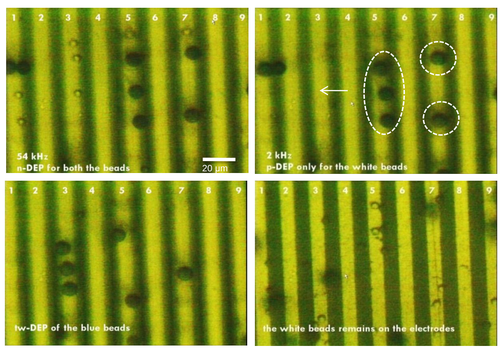


***Figure 11.** Bead separation: the white 5µm beads are retained on the electrodes while the blue 10µm beads laterally move.*

4. Conclusions and future works

This work presents two modules in terms of both design and microfabrication technology: a MEA for single cell transfection and two IDEs arrays for the controlled delivery of bio-chemical species. They have been individually characterized. Preliminary electrical results have demonstrated the possibility to use the MEA-based device to explore, without optical steps, the presence of cells over the chip surface by using electrochemical impedance spectroscopy. Also the dielectrophoretic conveyance of microbeads performed by means of IDEs arrays has been proven. Our final goal will concern the realization of a full-integrated system in order to provide a fast and efficient platform for *in-vitro* drug screening. In particular, the future works of this study will concern the stimulation of cells adherent to electrodes and the control of single cell morphology variation analyzing the modulation of electrode/cell impedance. The same process will be applied to different chip chambers in order to perform different transfections by using the microfluidic paths.

Acknowledgements
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