ON-LINE MONITORING OF CYTOTOXIC EFFECTS USING EIS BASED CELL-CHIPS

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An important goal of biomedical research is the development of tools for high throughput evaluation of drugs effects and cytotoxicity tests. In this respect, electrochemical impedance spectroscopy (EIS) is an emerging technique for the fabrication of sensitive and specific biosensors and lab-on-chips since the immobilization/adhesion of biomolecules or cells on biofunctionalized electrodes alters the capacitance C and interfacial electron transfer resistance R_{ET}. In particular, for cell layers, R_{ET} and C are correlated to cell viability, adhesion and cytoskeleton organization and this approach has been demonstrated to be a successfull strategy to monitor cell behaviour (cells micromotion, cells attachment and spreading, cell concentration and growth or apoptosis [1-5]). In the future such EIS devices are expected to replace *in vivo* tests on animals.

Here we demonstrate EIS cell chips able to monitor cell growth, morphology, adhesion and their changes as a consequence of treatment with drugs or toxic compounds. As a case study, we investigated the uptake of copper ions and its effect on HeLa cells. For further understanding, we also carried out in parallel an AFM characterization of cells and Cu effects and monitored them in real-time using an inverted microscope during the EIS experiments.

Specifically, our chips consist of a cell culture chamber made of PDMS with integrated interdigitated electrodes (with a line-space period of 40 μm and covering a 2 x 2.5 mm^2). ITO and Cr/Au (respectively 100 nm and 3/10 nm thick) electrodes were fabricated by optical lithography, lift-off and etching on glass substrates. The PDMS cell culture chamber was realized by replica molding from a hard master. The whole device is made using transparent (or semitransparent) and biocompatible materials (fig. 1) in order to be mounted on an inverted microscope for real-time monitoring of cells during measurements to correlate cell growth, status and detachment to changes in the EIS signal.

In fig. 2, we show typical AFM images of HeLa cells in physiological conditions **(a)** and after treatment with copper ions (500 μM) for 2h and 4h **(b-c)**. Experimental results indicate that at the beginning of the treatment the toxic effect of copper causes a spreading of cells (they become thinner, fig 2b). Then after a longer treatment some of them start to acquire a round shape (see for example the cell indicated by narrow) and detach as a consequence of cell death. These cytotoxic effects can be easily identified in our chips. Both the Nyquist and Bode plots reported in fig.3 change significantly. Cell attachment and growth onto the electrodes induces an increasing impedance compared with the empty device. Looking at EIS spectra (Nyquist plot in fig 3a) is possible to distinguish two different semicircles (not present in the empty device). The semicircle at higher frequencies can be ascribed to round shape cells, while the portion at lower frequencies to adhering and spreading cells into the same device. The equivalent circuit is shown in fig3b, where the Warburg impedance Z_W consists of a resistance R_W and a capacitance C_W in parallel. During the treatments R_W increases from 50 to 160 kΩ while C_W decreases from 330 nF to 180 nF.

In conclusion, these results reveal that our cell chips provide an easy and real-time tool to study cells attachment/spreading and to perform viability and cytotoxicity tests. They are cheap and reusable and join a great sensitivity and low cost both for fabrication and usage, since they do not require any additional reagent. Moreover, they can be easily multiplexed to monitor in parallel the effect of different drugs/compounds. In the future such devices will be useful to perform drug screening without animal sacrifice and to achieve this goal we are integrating in such devices new modules for temperature regulation and drug delivery.

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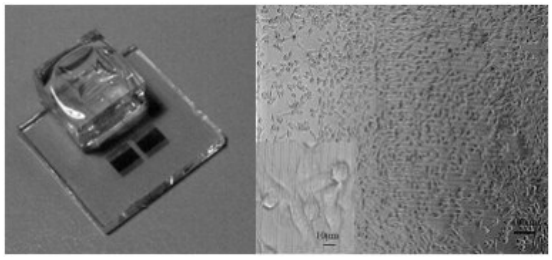

Fig. 1: Adhesion of Hela cells on ITO interdigitated electrodes on glass and picture of the whole device.

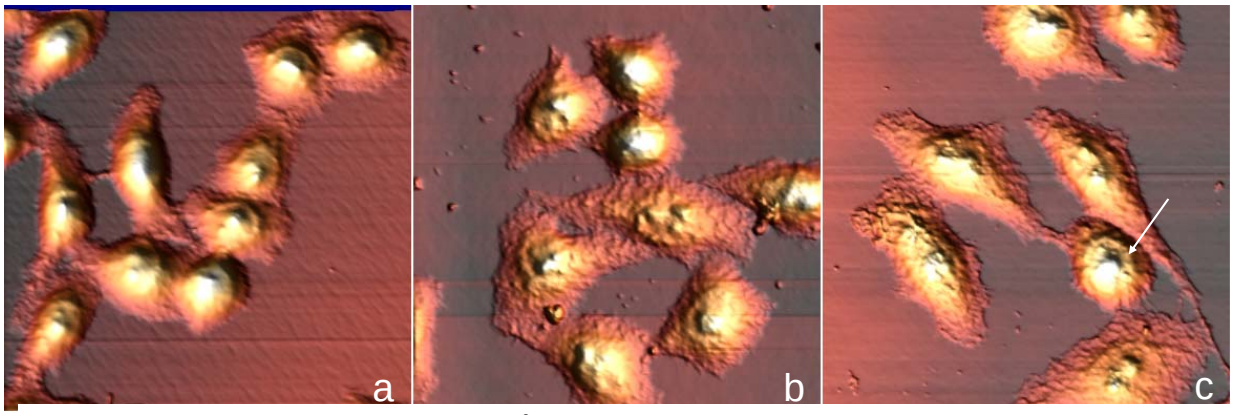

Fig.2: 3D AFM images (area 100 μm^2) of HeLa cells after different time of incubation with copper: **A**) control **B**) 2h at $CuCl_2$ 500µM **C**) 4h at $CuCl_2$ 500µM

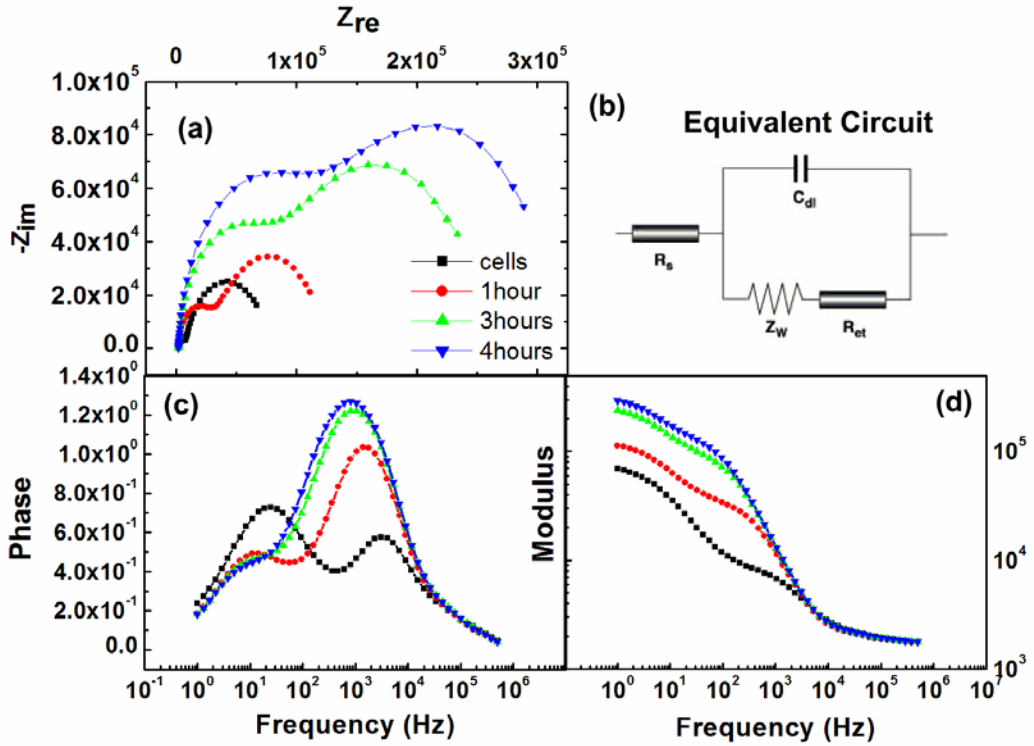

Fig. 3: Nyquist (Z_{re} vs Z_{im}) and Bode plots from chips with HeLa cells after treatment with copper for different time (0-4 hours). The data were recorded at 0V in culture medium in the presence of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1), 10 mM. The equivalent circuit is also reported in (b).