Multipurpose biochips - Toward on-chip medicine

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In recent years, miniaturization of biosensors and their integration in microarrays and functional biochips enabled massive parallel detection of analytes, diseases and disease susceptibilities, as well as identification of personalized drug response profiles. Examples of bioassays and biological procedures that have been miniaturized into a chip format include DNA sequencing, polymerase chain reaction, electrophoresis, DNA separation, enzymatic assays, immunoassays, cell counting, cell sorting, cell culture, high-throughput screening in drug development. In all these applications, the microfluidic approach brought new opportunities and capabilities. These progresses opened the way to novel devices and biochips which allow analyzing in parallel large numbers of biological molecules, cells and drugs. Another recent promising application concerns the detection of biological pathogens and disease-associated markers. Here the development of multipurpose impedimetric biochips with integrated microfluidic components is described [1-3].

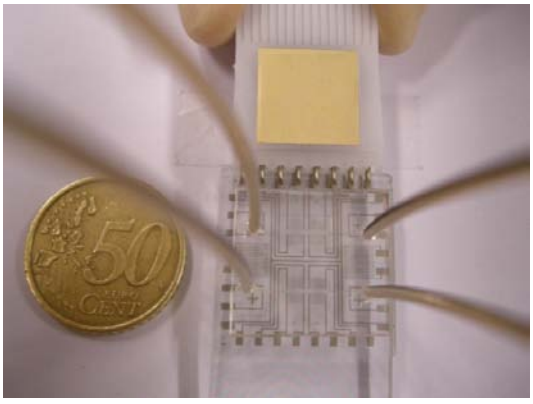

Figure 1. *Biochip layout with interdigitated electrodes, temperature sensors and heaters, PDMS microfluidic components (chambers with microchannels), inlet and outlet tubes.*

Gold interdigitated electrodes (with 10 µm spacing and width) were fabricated on glass substrates by optical lithography. Each device consists of two sensing areas, both including four interdigitated electrodes to allow statistical analysis or microarray detection (Figure 1). Microfluidic chambers with their own inlet and outlet microchannels were fabricated by replica molding from a hard master made by a 200 µm height SU-8 photoresist. To assemble the whole device a step of oxygen plasma exposure of PDMS (20 minutes at 0.5 mbar) and a rapid cleaning of the glass with the electrodes in piranha solution were carried out.

As a first application, microfluidic chips for flow immunoassay and ultrasensitive cholera toxin detection are described [3]. Cholera toxin is an enterotoxin of *Vibrio cholerae,* composed of two subunits, A (27 kDa) and B (11.6kDa) [4]. As highlighted by the World Health Organization, cholera is a major threat to human health which is becoming increasingly important as new major outbreaks continue to occur worldwide, especially in the wake of climate changes or where living conditions are crowded and water sources unprotected. The lethal dose for Cholera toxin in human is relatively low (~ 250 µg/kg), but rapid medical treatment can avoid death. So, there is a clinical need for the development of accurate, simple and rapid methods for detecting the bacterially-secreted toxin with high sensitivity. In particular, field use without the need of skilled operators is of extreme importance for developing countries.

Conventional methods for detection mainly address the cholera toxin subunit B (CT) and are based on recognition between cholera toxin and either gangliosides or specific antibodies by (for example) fluorescence, chemiluminescence or colorimetric detection. However, electrochemical transducers are the most interesting since they provide an easily processable response. Here a flow-injection impedimetric immunosensor for the sensitive, direct and label-free detection of cholera toxin is reported. Specifically, our strategy consisted in the immobilization of anti-

cholera toxin antibodies on gold electrodes through a number of functionalization steps, including mixed self assembled monolayers of 11-mercaptoundecanoic acid and 2-mercaptoethanol, protein A attachment and BSA passivation to saturate unbounded sites before antibody immobilization (Figure 2top). Impedance spectroscopy is a very effective read-out approach since the electron transfer resistance is highly sensitive to electrode modifications, allowing detection of bio-recognition events with high sensitivity. As shown in Figure 2bottom, a regular increase in impedance values and the related diameter of the Nyquist plot with higher CT concentrations was observed. A limit of detection smaller than 10 pM was achieved, a value thousands of times lower than the lethal dose [3]. The developed chips fulfil the requirement of low cost and quick reply of the assay and are expected to enable field screening, prompt diagnosis and medical intervention without the need of specialized personnel and expensive equipment, a perspective of special relevance for use in developing countries. Compared to literature, our microfluidic platform for sample handling allows to exploit the advantages of flow immunoassays in terms of quantitative results, speed, reduced sample handling and cost. Neither fluorescent tagging of reagents nor complex instrumentation and/or skilled operators are necessary.

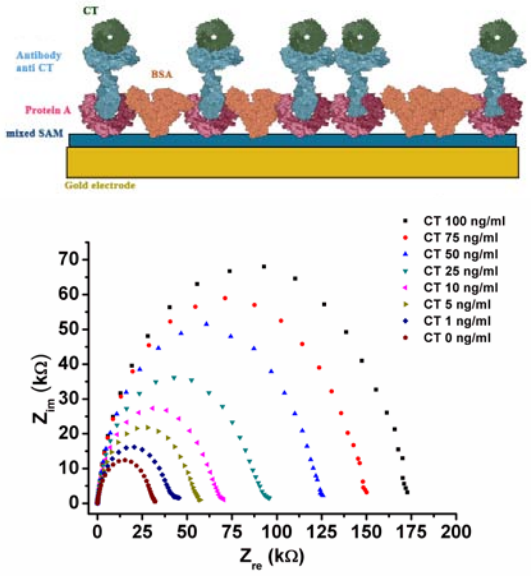

Figure 2. (top) *Schematic representation of electrode functionalization.* ***(bottom)*** *Nyquist spectra at decreasing CT concentrations.*

As a second application, cell chips to monitor cell migration are described [2]. Cell migration is a complex multistep process which plays a key role in many biological phenomena such as immune response, embryonic development and tissue repair. Importantly, its deregulation contributes to several diseases including tumor angiogenesis and metastasis. Cell motility has been thus recognized as a target for cancer therapy but the development of anti-cancer drugs is largely dependent on research on migration process using *in vitro* model systems. In this respect, however, conventional techniques (such as wound healing assays or transwell assays through a modified Boyden chamber) are based on microscopic observations leading to results strongly dependent on the investigators. In addition, these methods are manual and labor-intensive and are not suitable for high-throughput applications such as intensive screening for anti-migratory drugs. To overcome these limitations, in the last years significant efforts have focused on the development of *on-chip* cell migration assays.

Here we describe the development of new functional tools for automatic impedimetric transwell assays able to detect cell migration and investigate the influence of the microenvironment [2]. The fabricated biochips were inspired by a traditional modified Boyden chamber and consist of two compartments separated by a porous membrane (Figure 3 top). This structure (PDMS based) was bonded to an array of EIS sensors consisting of four different couples of interdigited electrodes. Cells introduced in the upper chamber through microfluidic channels reach after migration the lower compartment where they adhere to the electrodes, which allow detection of migrated cells by impedance measurements.

As proof of concept, the performance of our cell-chip was tested by investigating the migratory ability of hepatocellular carcinoma (HCC) cells as a function of microenvironment. HCC is one of the most common malignant tumors worldwide. In this respect the microenvironment around HCC cell lines and in particular the extracellular matrix (ECM) strongly influences differentiation, proliferation, gene expression and metastatic potential. ECM surrounding hepatocyte and its remodeling play an important role in the development of HCC. Tumor invasion and metastasis require the ability of tumor cells to interact with ECM through cell adhesion molecules on the cell surface. HCC showed significantly higher rates of adhesion to collagen

IV, laminin and fibronectin (Fn), thus the composition of microenviroment alters the invasive ability of HCC cells [5-7]. For this reason we challenged HCC cells to migrate on different extra-cellular matrix (ECM) components including laminin 1, collagen IV and laminin 5. In particular we tested three different cell lines (two invasive and one non invasive) in accordance with previous reports. All of the HCC cell lines, both invasive and noninvasive, were able to migrate on Coll-IV and Ln-1 resulting in impedance values between 40 and 130 kΩ. Alexander cells were found to migrate more easily on Coll IV than in presence of the other ECM proteins. On the contrary Alexander exhibit not significant migratory ability in presence of laminin 5. However, the invasive cell lines HLE and HLF migrate better on Ln-5 than on Ln-1 and Coll-IV as demonstrated by the larger values of R_{et} measured (200-300 kΩ). These results reveal that our cell chip provides reliable results that consistently overlap with those obtained with traditional standardized Boyden chambers (Figure 3). Thus, our biochips provide a new, easy tool to study cell migration and to perform automatic assays. This approach is easier and faster than traditional transwell assays and can be suitable for high-throughput studies in drug discovery applications.

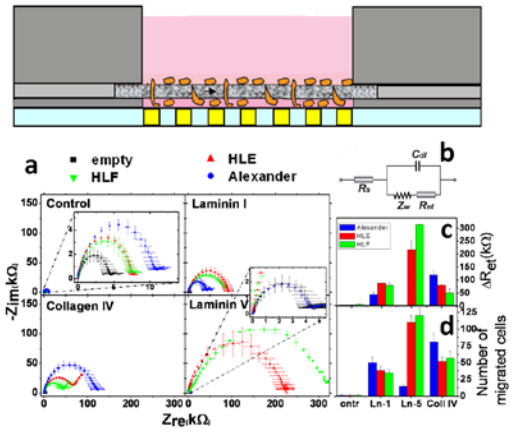

Figure 3: (top) *Structure of the developed cell chips for automatic EIS migration assays.* ***(bottom)*** *Influence of ECM on HCC cell migration.* ***(a)*** *Nyquist plots recorded after HCC cells migration on Ln-1, Coll-IV and Ln-5 and without any ECM protein as negative control. Error bars represent standard deviation calculated on measurements performed with different electrodes.* ***(b)*** *Equivalent circuit for impedance spectroscopy measurements.* ***(c and d)*** *Comparison between results of haptotactic assays performed with our EIS cell-chips (**c**) and traditional transwells [7] (**d**).*

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