Multipurpose biochips based on electric transduction

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The development of high throughput, low cost tools for the evaluation of pathological and threatening conditions is a major aim for both medical diagnostics and environmental and food contamination monitoring. Lab on a chip platforms allow to combine biosensors with microfluidic components for sample preparation, resulting in major advantages.

Among transduction techniques, an electrical detection is desirable because of large-scale integrability, easy read-out and high sensitivity. In this respect, electrochemical impedance spectroscopy (EIS) represents a powerful technique which allows biorecognition events to be easily detected since the immobilization/adhesion of biomolecules on biofunctionalized electrodes alters the capacitance C and the interfacial electron transfer resistance R_{ET}. As molecular layers are deposited on the surface of electrodes (during functionalization or detection phases), electron transfer process is hindered and this results in an increasing of the recorded impedance.

Recently we reported on impedance biochips able to detect antigen-antibodies recognition events [1, 2] and perform cellular studies [3, 4]. However, beyond medical diagnostics, the potential of lab-on-chip for the agricultural field is also huge, for example for controlling food safety along the whole production chain. Despite the improved awareness for food safety and security worldwide, food borne illnesses caused by food allergies or by the ingestion of foods contaminated with pathogens and/or their toxins are still one of the major public health concerns worldwide. Portable devices, allowing on-site, early and multiplexed quantitative detection of food allergens and pathogens are therefore highly sought for biosecurity purposes. Recently, we started to investigate the applicability of our biochips in this field.

In a first study, aptamers were employed as biorecognition elements for detecting the presence of mycotoxins contamination from food samples and in particular ochratoxin A (OTA), a toxic and potentially carcinogenic fungal toxin produced by *Penicillium* and *Aspergillus* species and found in a variety of food commodities, which elicit necrosis, hepatitis and immunosuppressive effect. A label free detection was achieved through the immobilization of a specific DNA aptamer onto the surface of gold trasducers with a dedicated functionalization as shown in Figure 1a, where an increase in the electron transfer resistance from 1 kΩ (after aptamer immobilization) to about 3 kΩ (after OTA recognition) can be observed. More studies are in progress to improve sensitivity.

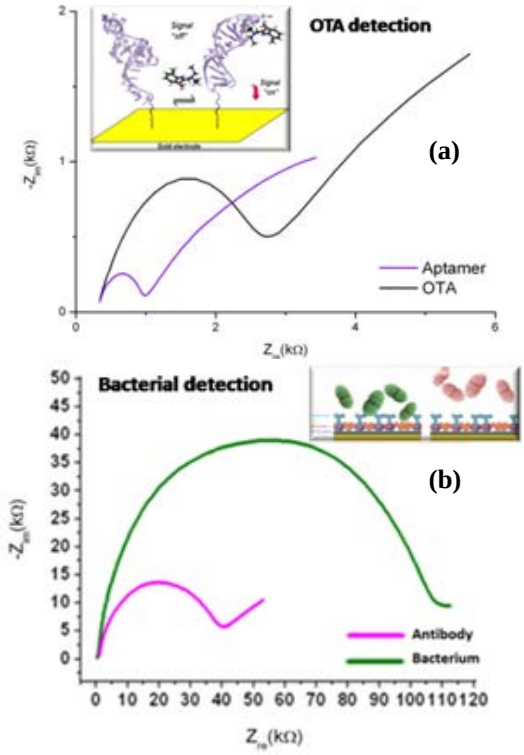

Figure 1. (a) *Nyquist plot related to biochips functionalized with aptamers (violet curve) and tested against samples containing OTA (black curve).* ***(b)*** *Nyquist plots acquired during L. monocytogenes detection: the purple curve corresponds to the impedance value of the antibody alone while the green curve corresponds to a sample with L. monocytogenes.*

Another application deals with the detection of some of the most common bacteria involved in foodborne diseases such as *Listeria monocytogenes* and *Staphylococcus aureus* by using specific antibodies against antigens expressed on the bacterial cells surface in order to immobilize the whole bacteria on the electrodes (Figure 1b). Using our biochips, we were able to quantitatively detect these bacteria in serial diluitions of thermally inactivated *L. monocytogenes* and *S. aureus.* No significant increase of the signal was obtained with negative controls of stationary phase broth cultures of pathogenic bacteria other than *L. monocytogenes* and *S. aureus* nor with a fresh sausage homogenate in which the absence of *L. monocytogenes* and *S. aureus* was previously ascertained by using the reference ISO method. So no unspecific interactions were relived even in presence of a complex and heterogeneous food matrix with a high background microflora. Foodborne diseases remain responsible for high levels of morbidity and mortality in the general population, but particularly for at-risk groups, such as infants and young children, elderly and immunocompromised people. With a low detection limit of as few as CFU/mL, our platform present important advantages. More studies are in progress for publication.

We also investigated potential applications in the field of food allergies, demonstrating an efficient detection of components involved in the common celiac disease (CD) [5]. For CD patients, the ingestion of food containing gluten can lead to unpleasant consequences ranging from mild cutaneous rash to more severe allergic reactions with massive production of IgE against gliadin which represents the toxic portion in gluten. The only solution is a gluten-free diet, but this is a challenge for both food producer companies and CD patients, since accurate attention must be paid constantly in everyday life and in production lines. Contaminations may occur, so the availability of portable, fast tools able to establish gliadin/gluten content from both liquid and solid matrixes can strongly facilitate everyday life. Our gliadin immunochip provides a robust platform for the quantification of gliadin concentration in commercial foodstuffs. This was demonstrated using beers and flours formulated for children nutrition and weaning, independently from their liquid or solid matrixes. For this study, EIS immunochips were functionalized with antibodies against gliadin. As a result, we were able to sensitively quantify the amount of antigen gliadin on its specific antibody. The achieved limit of detection of 0.5 µg/ml (data not shown) for gliadin is 20 times lower than the worldwide limit allowed for gluten-free food and largely under the limits reached with standard techniques. Moreover our biochips are faster and cheaper than currently employed ELISA-based methods. These characteristics along with portability and multiplexing possibility make the developed biochip a robust and promising resource for food producers, CD affected people or for children with metabolic troubles.

Finally, during this year we also investigated alternative electrical transduction approaches, in particular a method based on magnetoresistive (MR) sensors [6], whose advantage lies in the high sensitivity due to the absence of interfering signals when analyzing biological samples. However, differently from the EIS approach, labeling is necessary here by mean of magnetic markers. The surface of magnetic particles was enabled with carboxyl groups to facilitate the conjugation with biological molecules through the reaction with an amino group. The magnetic properties of the particles were also exploited to precipitate the conjugates with a magnet to remove the non-reactive molecules. The MR sensors were functionalized with 3-isocyanatopropyl- triethoxysilane in order to introduce functional groups on the surface which are reactive with the capture probes allowing their immobilization. For DNA hybridization and recognition on the MR sensors, a system with three probes was used. A capture probe was immobilized on the surface of silicon oxide protecting the MR sensors. A second DNA strand, signaling probe, was linked to the beads. The third target DNA in solution acts as a bridge connecting the other strands and resulting in the immobilization of the magnetic markers close to the MR sensors. Capture and signaling probe are complementary to two different regions of the target as shown in Figure 2a. Two devices were prepared, one with hybridization and another for reference measurements without hybridization. After silanization MR sensors were functionalized with DNA capture, then the devices were incubated with target DNA for hybridization. Later, the sensors were incubated in a solution containing the magnetic beads functionalized with DNA (the signaling probes). The magnetoresistance value evaluated for the MR sensors by applying in plane magnetic field was found to be 6 % as shown in Figure 2b. Since the sensor responded to in plane field, an out-of-plane magnetic field was thus applied to avoid sensor saturation by the magnetizing field. In Figure 2c, the difference in the voltage drop measured, as a function of applied out of plane magnetic field, across the hybridized and reference devices is plotted demonstrating a good sensitivity.

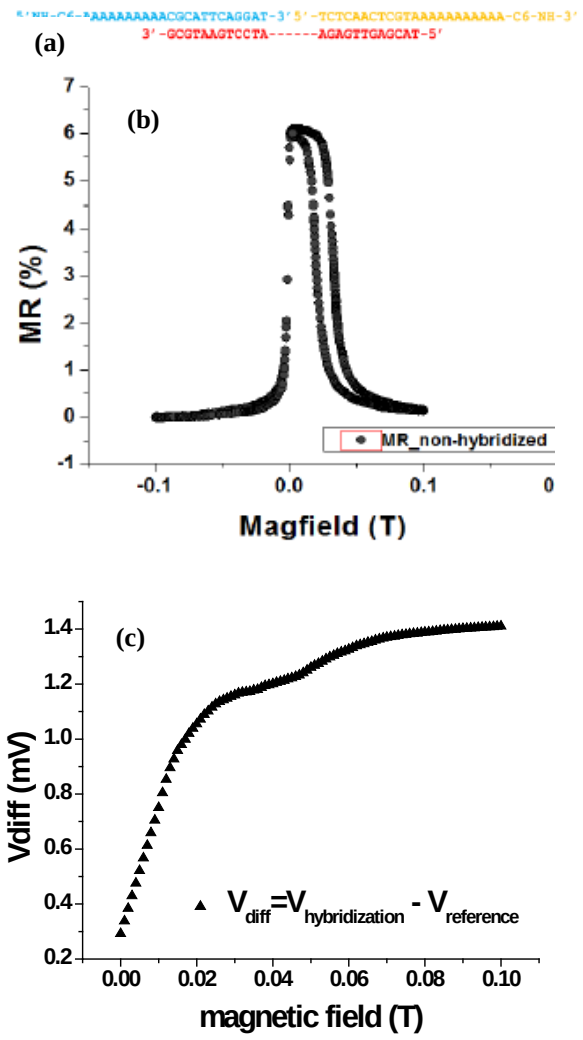

Figure 2. (a). *System with three DNA probes: capture, signaling and target DNA.* ***(b)*** *MR curve of the reference sample without hybridization reaction.* ***(c)*** *Voltage difference between sample with hybridization and without hybridization.*

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