A Multipurpose Biochip for Food Pathogens Detection

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The ingestion of food contaminated by pathogens and/or their toxins represents a serious burden for public health worldwide, being a major cause of morbidity and mortality. Unsafe food is responsible for more than 200 diseases and about 2 million deaths annually, including many children[1,2]. Moreover, the reported cases of food-borne illnesses and intoxications increased over the past decades despite a great attention[3].

Within this frame, *L. monocytogenes* and *S. aureus* strains represent two of the most common food-associated pathogens. The first one is a gram-positive bacterium and a facultative intracellular pathogen able to cause several clinical manifestations including meningoencephalitis, bacteriemia, abortion and gastroenteritis with a 24% case-fatality rate that increases to 75% in elderly, immunocompromised adults and pregnant women[4]. Listeriosis outbreaks have been linked to the consumption of different types of contaminated foods including soft cheeses, meat, fermented sausages, poultry, seafood and vegetable products[5, 6].

On the other hand, *S. aureus* is one of the most causative agent of bacterial diseases and food-borne intoxication worldwide, because of food poisoning caused by staphylococcal enterotoxins[7].

Appropriate diagnostics is thus crucial to assess the compliance of the foodstuffs with the European regulation. The diffusion and threat as biological weapons (incapacitating agent) of these two pathogens prompt the need for diagnostic devices.

To address this problem, we have developed a microfluidic lab on chip platform able to detect *L. monocytogenes* and *S. aureus* using a complex food matrix such as pork meat as model food. Our lab on a chip consists of a microfluidic platform with electrochemical impedance sensors functionalized with antibodies specific for *S. aureus* and *L. monocytogenes* and loaded with dilutions of bacteria stationary phase-broth culture.

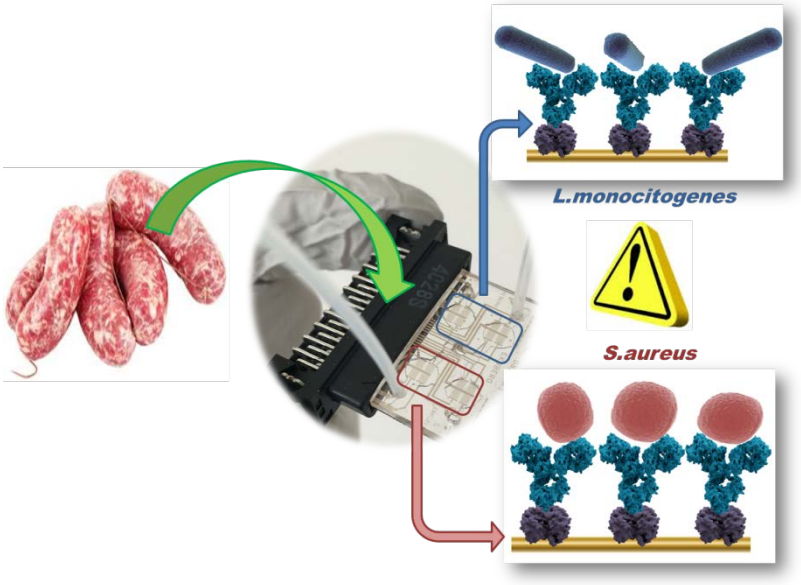

Figure 1: General scheme of the biosensing platform: the meat sample is injected into the device **(a)** where the electrodes arrays areas are functionalized with specific antibodies which recognize *S. aureus* and *L. monocytogenes* membrane antigens respectively

The baseline values for the immobilized antibodies were evaluated by impedance spectroscopy. Contributions of 42 kΩ for anti-*L. monocytogenes* and 45 kΩ for anti-*S. aureus* antibody layers were established, since their absorption on the electrodes quenches charge transport and increases the impedance. Then, a calibration curve was recorded for both pathogens under investigation, based on the same principle. In the case of thermally inactivated *L. monocytogenes,* we obtained a quantification for bacterial suspensions in the range from

7.5 x 10^8 down to 5 CFU/ml with the minimum revealed concentration corresponding to an impedance value of 70 kΩ. In the case of *S. aureus* the detection was possible in the range from 10^9 down to 1 CFU/ml, measuring a value of impedance of 51 kΩ for the lower concentration tested which corresponds to an increase of 6 kΩ over the anti-*S. aureus* baseline.

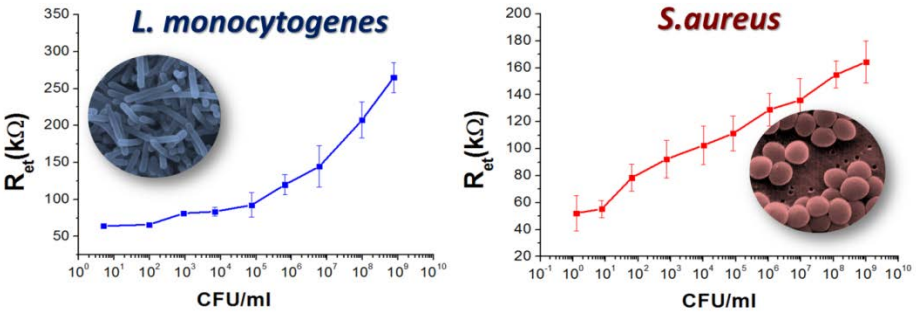

Figure 2: calibration curves for suspensions with known concentrations of *L. monocytogenes* and *S. aureus*. For *L. monocytogenes* **(a)** an impedimetric detection down to 5 CFU/ml was achieved. In the case of *S. aureus* **(b)** a detection limit of 1 CFU/ml was reached.

After sensitivity, we evaluated also the specificity of the biochip in order to be sure that there was no cross-talk between the biochip sensing chambers functionalized with either the anti-*L. monocytogenes* or anti- *S. aureus* antibodies.

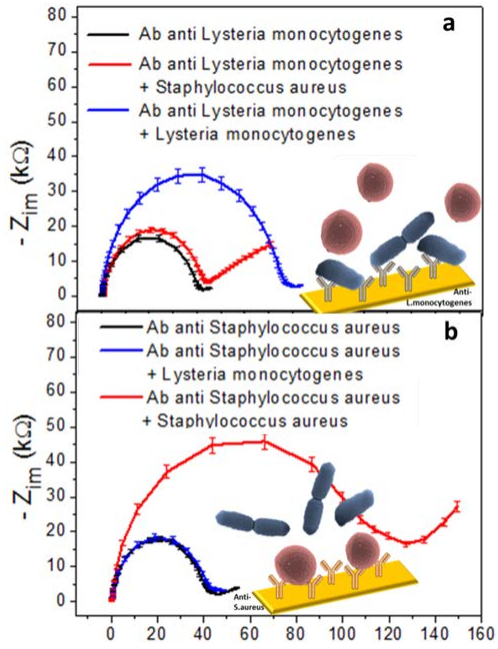

Figure 3: Specificity tests for anti- *L. monocytogenes* **(a)** and anti- *S. aureus* **(b)** functionalized chambers. Device was incubated with L. monocytogenes and S. aureus to perform the control experiments.

For this purpose, we incubated the chambers functionalized with anti- *L. monocytogenes* with 10^4 CFU/ml of *L. monocytogenes* and 10^4CFU/ml of *S. aureus* separately (Figure 3a). The same experiment was performed in chambers functionalized with anti- *S. aureus* (Figure 3b). In both cases the measured values for the unspecific reactions were quite overlapped with those related to the associated antibody layer. Impedance values measured in the chambers where the specific biorecognition was allowed were 75 kΩ for *L. monocytogenes* and 130 kΩ for *S. aureus*.

To demonstrate applicability as a common tool for food safety and quality control, we then carried out real sample tests. As a first step, we measured the unspecific contribution of the meat matrix over the antibody layer. The sample selected for the analysis was a fresh sausage homogenate. The unspecific adsorption of matrix components on the functionalized electrodes led to only a small increase quantified in less than 5 kΩ in each sensing chamber (Figure 4). Thus, we proceeded by analyzing real matrix samples spiked with known concentrations of pathogens suspensions, namely 10^3 CFU/ml for *L. monocytogenes* and 10^3 CFU/ml for *S. aureus*. As a result (Figure 4), we obtained an impedance of around 65kΩ and 75kΩ, which is compatible with the calibration value related to the same concentration, demonstrating the high selectivity of our device.

In conclusion, we were able to quantitatively detect *L. monocytogenes* in a range from 7.52 x 10^8 to 5. 0 CFU/mL and *S. aureus* from 1.02 x 10^9 to 1,26 CFU/mL. No significant increase of the signal was obtained with negative controls of stationary phase broth cultures of two different pathogenic bacteria nor with a fresh sausage homogenate thus demonstrating that no unspecific interactions were relived even in presence of a complex and heterogeneous food matrix.

Reference

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