Immunochips for multi-pathogen diagnosis of lower genital tract infections

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Female vaginal infections are common conditions, which carry higher risks in developing countries where delayed diagnosis and treatment may lead to more severe disease [1]. The most common causes of vaginitis are vulvovaginal candidiasis and bacterial vaginosis, typically caused not by a single entity, but by different bacterial strains [2]. Usually symptomatology and standard analytical techniques are employed for diagnosis. In particular, microbial cultures are the gold standard despite a number of drawbacks, such as low sensitivity, long duration (at least 72 hours), and the need for culture media specific for the different strains, trained staff and appropriate instrumentation [3]. Recently, molecular techniques have been developed but nucleic acid amplification tests are not suitable for point-of-care because of their high costs, complexity, need of highly trained staff and complex equipment [4]. To overcome these limitations new tools are needed.

In this respect, we have developed an impedimetric Lab-on-chip (LOC) platform for multi-pathogen diagnosis of lower genital tract infections caused by *Streptococcus agalactiae, Chlamydia trachomatis* and *Candida albicans* in women. The immunochip reported here (Figure 1) is based on a modification of our previously developed platform able to detect antigen-antibodies recognition events [5, 6] and perform cellular studies [7, 8]. Specifically, the biochip integrates a microfluidic module with four separate sensing areas, each one containing an array of 4 gold interdigited microelectrodes. Biosensing capability was achieved by means of specific functionalization (Figure 1d) of the microelectrodes [9] with monoclonal antibodies: chamber 1 with anti-*Streptococcus agalatctiae* antibody, chamber 2 with anti-*Chlamydia trachomatis* antibody, chamber 3 with anti-*Candida albicans* antibody, while the last chamber was not functionalized with any antibody in order to provide a negative control. The antibodies were chosen for their ability to recognize specific membrane proteins of the three above-mentioned pathogens. In this way, each chamber gives information about the infection of a specific pathogen and co-infections can be detected as simultaneous signals coming from different chambers.

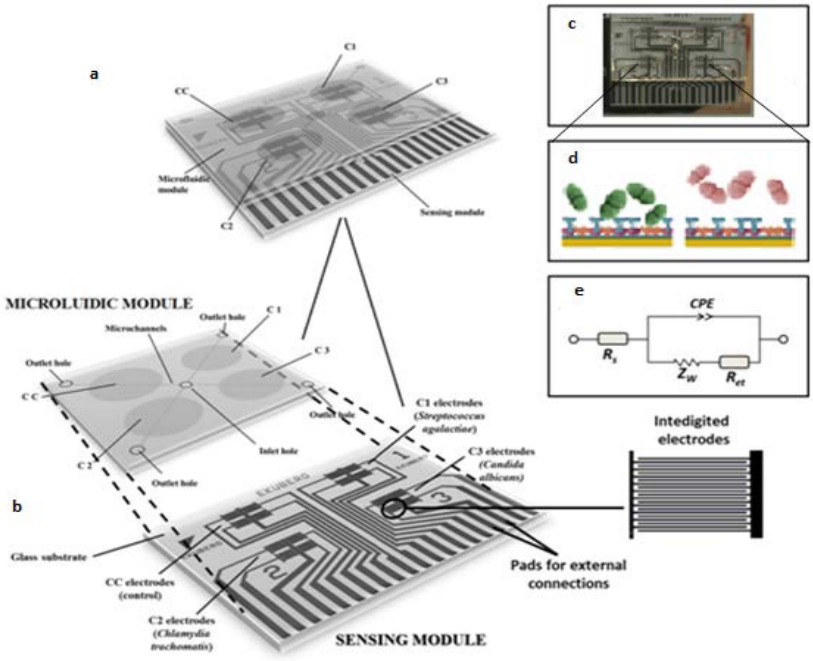

Figure 1. *Scheme of the immunochip, including its components and equivalent circuit.*

After immobilization of *anti-S. agalactiae* antibodies, impedance values of around 30 kΩ were reproducibly measured. In contrast, anti-*C. thrachomatis* and anti-*C. albicans* gave impedance values of about 40 kΩ. The impedance after BSA immobilization (the last step before antibody deposition) was around 20 kΩ. All these values were assumed as the baseline for subsequent experiments with test samples [9]. Then, to evaluate biochip sensitivity and specificity, three suspensions were prepared, each containing cells picked up from a colony of the pathogen under investigation in 1 ml of PBS. These suspensions were separately injected into different devices and incubated for 1 h. As shown in Figure 2, an impedance increase from 30 kΩ to 115 kΩ was observed with a suspension of *S. agalactiae* in the chamber functionalized with the anti-*S. agalactiae* antibodies (red curve in Fig. 2A), whilst in the case of *Candida albicans* the pathogen suspension caused an impedance increase from 40 kΩ to 100 kΩ in the chamber functionalized with the anti-*C. albicans* antibodies (green curve in Fig. 2B). In both cases, a negligible increment was measured for the three unrelated chambers, demonstrating very low unspecific signals. With *C. trachomatis* (Figure 2C), the impedance signal increased from 40 kΩ to 85 kΩ on the specific anti-*C. trachomatis* antibodies, with lower impedance increments in the other chambers. As a control, we tested the signals of all four chambers after incubating with a PBS solution in the absence of any pathogen (Figure 2D). This produced the same values as obtained after functionalization (about 35 kΩ for anti-*S. agalactiae* and about 40 kΩ for anti-*C. trachomatis* and *anti-C. albicans)*, showing the absence of unspecific signals.

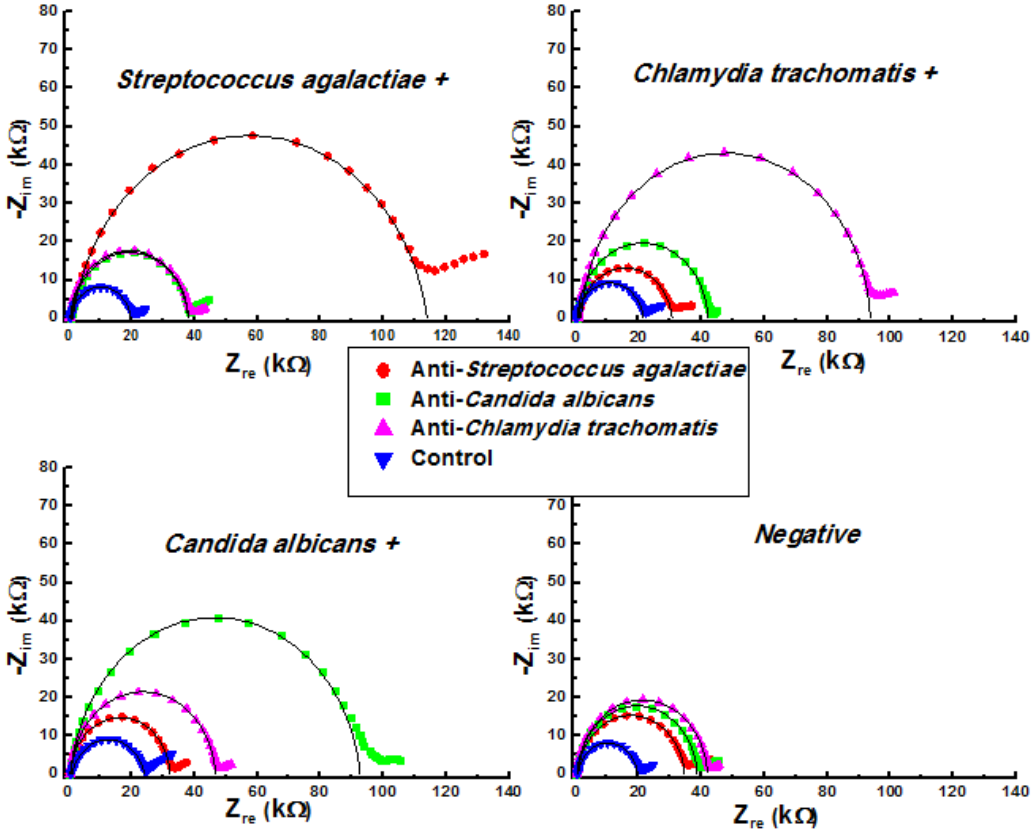

Figure 2. *Nyquist plots for immunochips characterization with a sample containing Streptococcus agalactiae* ***(A)****, Candida albicans* ***(B)****, Chlamydia trachomatis* ***(C)*** *and without pathogens* ***(D)****. Black lines are the fitting curve on the basis of equivalent circuit elements*

To demonstrate the diagnostic value of our biochip, a blind test on patient samples was also performed. Specifically, 45 samples derived from vaginal swabs were resuspended in sterile PBS and analyzed by injecting 90 µl in the microfluidic module with a micropipette (or by an insulin syringe). Standard microbial cultures were performed in parallel as a control. Based on some preliminary tests on vaginal samples (data not shown) and considering the contribution of the biological matrix, we defined a result as positive

when the impedance signals deriving from each chamber exceeded a threshold respectively of 45 kΩ for *S. agalactiae* and of 65 kΩ for *C. albicans* and *C. trachomatis*. These values are higher than the antibodies' baseline, adjusted considering unspecific adsorption of components of the vaginal mucus.

The analysis of real samples confirmed the efficiency of our method for the diagnosis of *C. albicans* and *S. agalactiae*, since in all these cases biochip outcomes were the same obtained by culture tests. Moreover, to prove its high level of selectivity we report a case identified with standard selective medium as a "*Candida non albicans*" infection which was not confirmed by our immunochip result, demonstrating a species-specific recognition ability of the device. Our sensing platform was also able to detect co-infections: in three samples containing both *C. albicans* and *S. agalactiae*, an impedance increase was detected from both the corresponding chambers, while no change was recorded from the negative control and the anti-*C. trachomatis* chambers.

In conclusion, the developed microfluidic platform is suitable as a multiplexed immunoassay overcoming the much longer analysis time and higher cost of standard techniques. Moreover our device may be readily improved by expanding the number of sensing areas and hence of the detected pathogens within the same biochip, thus increasing its diagnostic power. This, in combination with portability, makes our platform a promising *point of care* device for gynecologists and patients which would not only reduce the number of invasive examinations and tests, but also minimize analysis time and costs. These features are likely to be of particular value in developing countries where lower genital tract infections are highly prevalent and the health care system lacks adequate medical and economical resources.

These studies led to a patent [10] and a publication [11].

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