On chip screening for Prostate cancer: an EIS microfluidic platform for contemporary detection of free and total PSA

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Prostate Cancer (PCa) is a very common disease affecting at least 2 millions men in the United States and another 4 millions in Europe. One of the main problems is the lack of specificity of the serum marker PSA (Prostate Specific Antigen), a glycoprotein of about 34 kDa [1-4] which is detected in routine blood tests, since several abnormalities (including benign hyperplasia) can lead to increased serum concentrations [5]. Thus other investigations are often recommended such as DRE (Digital Rectal Examination), TRUS (Trans-Rectal Ultrasonography) or biopsy, which however are highly invasive and cause discomfort to patients, increasing themselves the levels of serum PSA, and modifying the integrity of the gland.

In this work a new sensing platform is presented for the contemporary on chip detection of two PSA forms (free and in complex with α-1-antichymotrypsin together referred as total PSA) [6]. Our prototype is based on impedance spectroscopy without the need of dedicated instruments like microtiter plate readers. Similar impedance biochips have been already demonstrated to be able to detect cellular behaviour in response to a chemical stimulus [7, 8], cell migration [9] and bio-recognition events between immobilized antibodies and related antigens in aqueous solution [10].

Specifically, each biochip integrates a microfluidic PDMS (Polydimethylsiloxane) platform with two separate sensing areas containing transducer arrays of gold interdigitated microelectrodes, having inlet/outlet microchannels for fluid handling. The microelectrodes (with 10 μm spacing and width) were fabricated on glass substrates by optical lithography and lift-off, while the microfluidic module was realized by replica molding with a chamber volume of 28 μL (7mm x 4 mm x 100 μm height) connected with its own inlet and outlet microchannels.

The two sensing areas were functionalized with antibodies directed respectively against freePSA and totalPSA and employed to evaluate their percentage ratio in a sample where they are both present. Antigen-antibody interaction was detected as a change in capacitance and

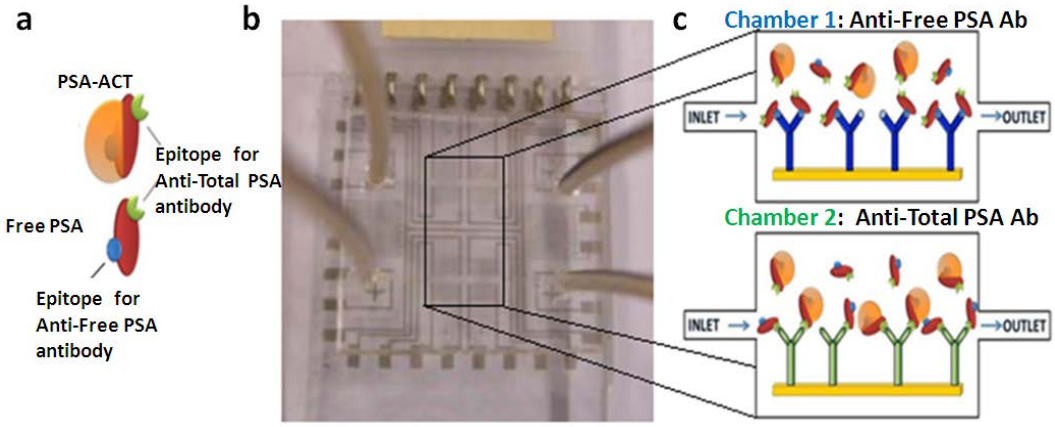

Figure 1 (a) *Schematic representation of PSA antigens-related (PSA-ACT and freePSA) and* ***(b)*** *device composed of two chambers for antigens detection:* ***(c)*** *one is functionalized with antibodies Anti-freePSA (Chamber 1) and the other one with antibodies Anti- totalPSA (Chamber 2).*

interfacial electron transfer resistance due to the formation of bound complexes on the electrode surface. The two antibodies employed recognize two different epitopes of PSA: in particular Anti-freePSA antibody captures PSA thanks to a defined domain of the protein which is instead masked in PSA-α1-antichimotrypsine complex form (PSA-ACT), so that antibody anti-freePSA is highly specific for its own antigen. On the contrary antibody anti-totalPSA is directed toward a portion of PSA which is always accessible both in freePSA and in PSA-ACT, so that both these two PSA forms can be detected with the same antibody to give the amount of total PSA (Figure 1). The percent ratio between amounts of freePSA and totalPSA measured in the two biochip chambers is the value needed to discriminate between a condition of malignancy or benign prostatic hyperplasia and can be evaluated by combining results coming from the two sensors arrays.

The first step in the optimization of the PSA chip was obtaining a proper calibration for each antibody by flowing a solution with known concentrations of freePSA and PSA-ACT (Figure 2) [6]. Then, the next step was testing two different mixtures of freePSA and PSA-ACT. For this purpose, two solutions were prepared, both corresponding to an uncertain diagnosis on the basis of a simple total PSA evaluation with a total PSA amount comprised in the 4-15 ng/ml range. Specifically, fixing a diagnostic cut-off of 25% [11-13], the first mixture had a 20% freePSA/totalPSA ratio corresponding to a suspected PCa, while the second one contained an equal amount (50%) of freePSA and PSA-ACT to simulate a BPH case. In both cases the impedance values were recorded in each chamber.

Initially, chamber 1 was employed (Ab Anti-freePSA) and the baseline (around 27 kΩ) due to the presence of the Anti-freePSA antibody on the electrodes was subtracted from the recorded impedance (R_{et} measured) in order to evaluate the freePSA contribution to impedance ΔR_{et} whose value was then interpolated in calibration curve 1 (freePSA on Ab Anti-freePSA) to obtain the effective amount of freePSA in the mixture (2.6 ng/ml

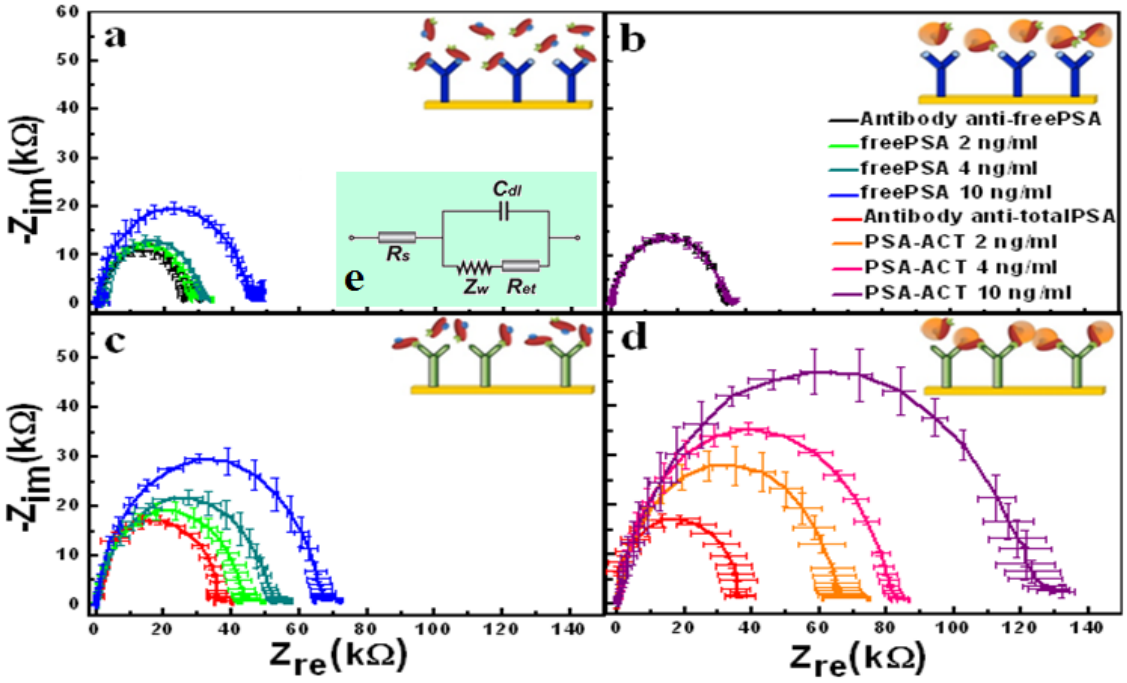

Figure 2 *Electrochemical Spectroscopy analysis on chip.* ***(a)*** *Nyquist spectra for 2, 4 and 10 ng/ml of freePSA on Antidodies Anti-freePSA layer.* ***(b)*** *Negative control: no recognition between PSA-ACT and Antibodies anti-freePSA was recorded.* ***(c)*** *Nyquist curves for 2, 4 and 10 ng/ml of freePSA on antibodies anti-totalPSA.* ***(d)*** *Calibration for 2, 4 and 10 ng/ml of ACT-PSA on antibodies anti-totalPSA* ***(e)*** *Equivalent circuit for impedance spectroscopy measurements. The circuit includes ohmic resistance of the electrolyte solution R_s, Warburg impedance Z_w resulted from the ionic diffusion of the electrolyte, double layer capacitance C_{dl} and electron transfer resistance R_{et}*

in the first mixture). Successively chamber 2 was used with only a slight difference in baseline subtraction since beyond the Ab contribution also the freePSA contribution has to be removed in this case when calculating the impedance ΔR_{et} related to PSA-ACT. For this purpose, the previously estimated concentration of freePSA was employed to evaluate the second offset (11.6 kΩ) related to its signal by using calibration curve 2 (freePSA on antibodies Anti-totalPSA. The value resulting from the differences (94.5 kΩ) can now be compared to an expected value of 20% for the first mixture.

A similar analysis was performed on the second mixture where chamber 1 provided measured R_{et} = 37.3kΩ, baseline Ab 27.6 kΩ, ΔR_{et} = 9.8kΩ corresponding to a concentration of freePSA of 6.3ng/ml. On the other hand, chamber 2 gave the following results: measured R_{et} = 133.6 kΩ, baseline Ab 36.5 kΩ, baseline freePSA=21.4 kΩ resulting in ΔR_{et} = 75.7kΩ corresponding to a concentration of PSA-ACT of 8.7ng/ml. Thus in this case the nominal ratio is 50%, and the biochips revealed a 42% value. As a consequence, we conclude that biochips are able to distinguish among the two cases providing a useful diagnostic information and the ability to discriminate among a condition of PCa or BPH on the basis of the freePSA/totalPSA ratio.

In conclusion we demonstrated a microfluidic platform suitable for PCa screening in a diagnostic range which is suitable for further improvement by increasing the number of biomarkers within the same biochip to further enhance screening specificity and enable patients to avoid very invasive examinations and multiple tests before establishing diagnosis, administrating medical treatments or deciding for surgical intervention.

Concerning sensitivity, our biochips are competitive with most of label-free assays demonstrated in literature (limit of detection was found to be about 1ng/ml, a value similar to what we obtained for the detection of cholera toxin subunits [10]). In the case of label-based sensors limits of detection can be lower than in our system but these devices require many analytical steps (for example secondary label antibodies) which result in higher costs. Then, we still emphasize as no label-free platforms exist in literature which allow to evaluate the free-to-total PSA ratio.

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