

Multi-panel, on-single-chip Memristive Biosensing

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Abstract—Memristive biosensors have demonstrated excellent capabilities for ultrasensitive bio-detection. In the present work, memristive biosensing chips are designed, fabricated and implemented in a for the first time presented multi-panel on-chip detection for discrete sensing of a target molecule through separate functionalization of individual devices on the same chip. The biosensing scheme is validated by means of labeled (i.e. fluorescence) and label-free (i.e. electrical) characterization methods. This novel memristive multi-panel sensing paradigm paves the way for fast and ultrasensitive PoC (point-of-care) devices allowing the detection of specific targets in complex matrixes where non-specific molecules are present as well, and opening great potential for the application of memristive phenomena in multiplexed ultrasensitive bio-detection and theranostics.

Index Terms— Memristive Biosensors; Bio-functionalization; Multi-panel detection;

I. INTRODUCTION

SEMICONDUCTOR nanowires may provide low-cost microchips and therefore are considered as effective and highly promising building blocks for miniaturized bioassays dedicated to medical applications, in both diagnostics and therapeutics. In this framework, silicon nanowire-arrays that exhibit memristive electrical properties [1]-[3] are bio-functionalized with receptor molecules i.e. antibodies or DNA aptamers, giving rise to the so-called memristive biosensors. These memristive sensors have already successfully achieved ultrasensitive detection for cancer biomarkers, demonstrating atto-molar concentration sensing performance [4] as well as effective screening of therapeutic compounds, along with the possibility for continuous drug monitoring [5] therefore showing immense potential for ultrasensitive and precise biosensing. The bio-detection is based on the hysteresis

modification appearing in the electrical characteristics upon the introduction of charged biological species on the nanodevices' surface (bio-functionalization procedures and target molecule uptake) [6], [7], [8].

Moreover, the design and realization of memristive biosensing electronic platforms was realized and validated [9], [10] providing a fast, fully-automatized and simultaneous sensing output of multiple individual memristive biosensors on a single chip. Highlights of the development steps of memristive biosensors are summarized in Table I.

Table I. Developing Memristive Biosensors

Physical and Chemical Sensing	
Anti-rabbit	[6]
pH	[11]
VGEF	[12]
Prostate Specific Antigen (PSA)	[4]
Tenofovir (TFV)	[5]
Theoretical Study and Modelling	
Equivalent circuit modeling	[7], [13]
Physical modeling	[8]
Integration, optimization towards PoC schemes	
Bio-functionalization	[14]
Microfluidics	[15]
Electronic platforms	[9] [10]
Multi-panel on-chip sensing	<i>Present work</i>

Meanwhile, the importance of multi-panel detection in a lab-on-a-chip framework is even more pronounced when considering the aspect of theranostics applications where both disease biomarkers and therapeutic compounds are simultaneously monitored in order to reflect the effect of the treatment [16]-[19]. This scheme may involve target molecules that are competing or complementary to each other. The first case concerns for instance a disease marker and the specific therapeutic drug or its metabolites, evaluating the efficiency of the medical treatment in the form of a feedback loop, allowing appropriate adjustments for enhancing the therapy [20]-[22]. The case of complementary markers refers to simultaneous detection of multiple biomarkers indicating the same disease or disorder targeting at a more accurate evaluation of the disease evolution and providing enhanced diagnosis [23]. Furthermore, the discrete sensing of specific biomarkers when included in a more complex matrix or cocktail of multiple substances consists another significant challenge in biosensing.

In this work, a proof-of-concept study demonstrating multi-panel detection paradigm involving memristive biosensors and individualized sensing is for the first time presented and validated through electrical and fluorescence characterization techniques. This biosensing scheme paves the way for

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individualized biomarker sensing in a complex matrix, as well as for multiple biomarkers/drugs detection on the same chip and application in theranostics. Sensing chips of memristive biosensors conjugated with metallic extension electrodes are validated for successful sensing performance and then implemented for multi-panel sensing.

II. MATERIALS AND METHODS

A. Memristive Biosensing chips

Two-terminal silicon (Si) nanofabricated wire-arrays exhibiting memristive electrical properties are acquired through a top-down fabrication process using Silicon-on-Insulator (SOI) wafers as described in [7], by implementing electron beam lithography and Deep Reactive Ion Etching (DRIE). The wires are suspended and anchored between Nickel Silicide (NiSi) pads forming Schottky-barriers and serving for the electrical characterization of the nanodevices. Metal lines are integrated on top of the already fabricated nanostructures and serve as extension line electrodes to the NiSi pads of the devices, as described in [9]. The metal lines are designed taking into consideration the fact that the hysteretic properties of the memristive biosensors are sensitive to additional resistive elements introduced in series to the nanofabricated devices, eliminating or even completing masking the memristive properties of the nanodevices. For this reason, the extension lines design follows the outcomes of a relevant computational study and calculations [9], [13] for respecting the resistance tolerance of the system. A common source and separated drains are considered for individual measurement of twelve integrated sensors (as seen in Fig. 1) that allow possibility for adequate statistical analysis to determine average value and standard deviation. This statistical analysis is required for obtaining reliable results and overcome the variability of the sensors originating from the DRIE process. In addition, such realization of multiple sensors allows the possibility for simultaneous sensing (multi-panel sensing), that consists the main aim of the present study.

To confirm the sound functionality of the metal lines the memristive devices were functionalized by exposure of the surface to antibody against Prostate Specific Antigen (PSA), i.e. anti-PSA antibody (Abcam-ab10185), solution in Phosphate Buffered Saline (PBS) (pH 7.4 Sigma-Aldrich) for 4 h at room temperature, thoroughly rinsed with the same buffer and gently dried with N₂ flow. Then, the antigen uptake was performed through successive 45 min incubations of the sensing chip in solutions of PSA (Millipore Angebot R-1939458.1; 539834 purchased from Merck) in PBS. The measurements were performed with the prober needles placed on the Pt pads instead of the NiSi pads.

The electrical monitoring of the memristive nano-bio-sensors was performed utilizing the configuration consisting of a Cascade Microtech Probe Station in combination to a Hewlett-Packard 4165A Precision Semiconductor Parameter Analyzer. Fluorescence characterization is carried out using scanner Typhoon Trio (GE Healthcare).

B. Multiplexing approach for sensing

A single-chip, detection in a multi-panel mode is for the first time attempted implementing memristive biosensors. The

memristive devices included in the same 1 cm x 1 cm chip are first subjected to a silanization step with GPTES (440167, Sigma-Aldrich). Then the devices are separately bio-functionalized with different antibody solutions. Antibodies either from mouse, goat or rabbit were diluted within PBS to get a final concentration of 0.5 mg/ml with 0.5% trehalose (T9449, D-(+)-Trehalose dihydrate from corn starch=99%, Sigma). Multiplexed biosensing requires post-fabrication nanowire functionalization with probe molecules, (though robotic spotting [24], ink-jet printing [25], selective heating [26], electrochemically [27], or via dip-pen nanolithography [28]). In this work, a micro-spotter (GeSim NanoPlotter 2.1) is used for specific and separate bio-functionalization of each nanodevice with the different antibodies under consideration, with a drop of 400 pL antibody-solution of an initial diameter set at 150 μm. A rabbit antibody is used as a positive-control bio-functionalization reagent and is spotted to various devices of the sensing chip. Meanwhile, three different negative controls are taken into consideration: a mouse and a goat antibody both applied at the same concentrations as the positive control as well as plain buffer solution (PBS).

The micro-spotting procedure finally results in discrete bio-functionalization of different devices (Fig.1). Following the bio-functionalization the chip remains in a humid chamber for 1 h in order to ensure the attachment of the bio-functionalization reagents on the nanodevice surface. The additional humidity introduced, aims at the conservation of the liquid antibody-solution drops which are very sensitive to evaporation issues. In order to prevent non-specific binding of proteins during the detection measurement, the remaining active GPTES-derived groups are passivated by applying 10 mM ethanolamine in PBS solution at room temperature. Washing process is further performed to remove unreacted molecules.

An additional blocking step is carried out with PBS containing 3% gelatin from cold water fish skin. This further blocking forms a stable and specific receptor layer, due to the passivation property of neutrally charged gelatin molecules that aims to prevent the empty sites of the device active area from interacting with charged non-specific species from the analyte solution. Then, what follows is the antigen uptake through simultaneous incubation of all the nanodevices under

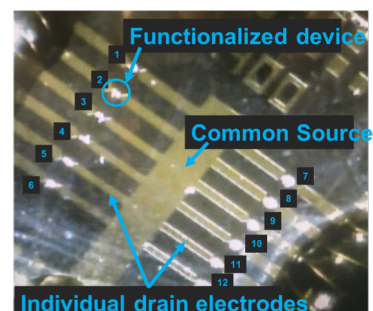


Fig. 1. Optical microscopy image depicting the selective spotting with receptor molecules. A closer view of the surface of the multi-sensing chip is shown, depicting the twelve sensors locations, that are integrated with metal lines. The discrete functionalization scheme is visible for each individual sensor and shown as a droplet located exactly at the sensor's regions. The memristive devices are integrated with the extension Pt electrodes in order to enable the electrical characterization as well as the integration with the electronic readout platform as reported in [9] and [10].

consideration for 45 min in humid environment. A pipette of 2 μL is used to efficiently transfer a sufficient amount of antigen solution directly and restricted at the region of interest i.e. nanowires' region. Two different aspects are considered through the antigen uptake. A selected region of the chip is incubated in a high concentration of antigen-tagged with fluorescent substance, destined for a fluorescence study and another region is incubated with successive 45 min incubations increasing antigen concentrations belonging to the range close and below the clinical range of most biomarkers, like for example PSA. Each incubation is followed by a washing step with gelatin, two washing steps with PBS and a final washing step with PBS 10% in order to remove any salts originated from the buffer.

III. RESULTS AND DISCUSSION

A. Performance of Memristive Biosensing chips

The electrical response using the fabricated chips is acquired before and after the bio-functionalization process and antigen uptake with increasing antigen concentration (Fig. 2). The obtained results clearly show that the hysteresis is successfully maintained and the voltage gap is acquired as expected upon bio-functionalization and decreases with the increasing antigen uptake as shown in Fig. 2.

B. Multi-panel sensing with memristive biosensors

For the multi-panel detection two separate methods (fluorescence detection and label-free sensing through electrical characterization) are independently implemented to verify the multi-panel sensing capabilities of the memristive biosensors.

1) Fluorescence Sensing

The results obtained from fluorescence characterization are illustrated in Fig. 3. It is depicted that selected bio-functionalization is successfully achieved and only the devices functionalized with the positive control antibody finally demonstrate the capability to bind the target antigens. Despite the fact that accurate bio-functionalization is achieved strictly

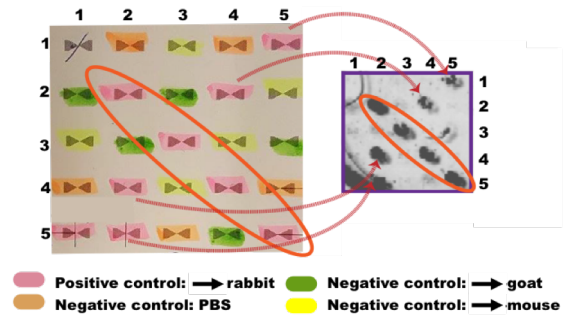


Fig. 3. Fluorescence results demonstrate the successful multi-panel bio-functionalization.

at the region of each suspended nanowire, due to adhesion phenomena occurring during the incubation time the bio-functionalization solution is slightly spread outside the target area and finally stabilized to a slightly larger droplet, after established hydrodynamic and hydrostatic forces equilibrium, as well as evaporation aspects.

2) Label-free sensing

The nanodevices, bio-functionalized with the different antibodies and the PBS, are electrically characterized (baseline measurement), followed by the electrical monitoring of the nanodevices with increasing antigen concentrations. The introduction of charged residues on the surface of the nanodevices, as it is expected, induces the appearance of a voltage gap in the semi-logarithmic current to voltage characteristics. Meanwhile, the binding of antigen (through the receptor-target molecule relation) introduces a masking contribution to the effect already brought by antibodies, resulting in a decreasing voltage gap. Indeed, the voltage gap presented at the electrical characteristics of the nanodevices functionalized with the positive control antibody demonstrates smaller values after the antigen uptake, verifying successful antibody-antigen binding (Fig. 4). However, no significant difference is recorder for the case of the negative-control bio-functionalized devices (Fig. 4). Further study of the analytical

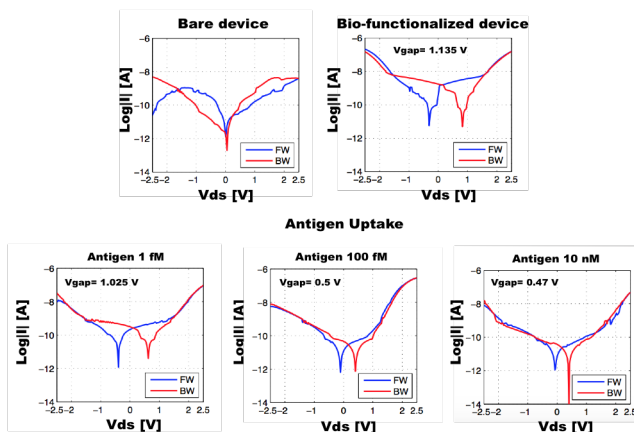


Fig. 2. Indicative semi-logarithmic current to voltage characteristics obtained for the same device measured at the Pt pads using the Probe Station and Keithley configuration. The different graphs correspond to the bare device, after the bio-functionalization with Ab and with increasing antigen uptake (from 1 fM to 10 nM).

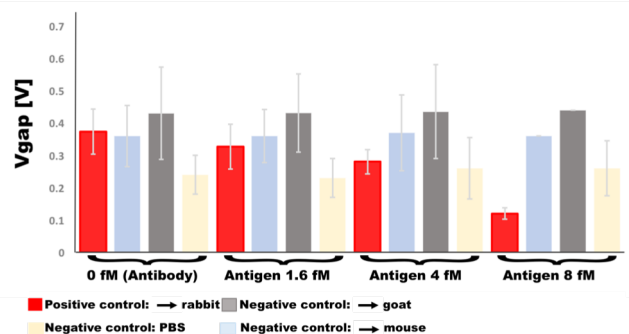


Fig. 4. Electrical characterization results bringing proof of the successful multi-panel bio-functionalization. The average value of the voltage gap acquired for increasing concentrations of four different molecules which are the target molecule and three negative controls. No significant signal difference is depicted for the case of the negative-control bio-functionalized sensors in contrast to the decreasing voltage gap trend with increasing concentration shown for the case of the target molecule.

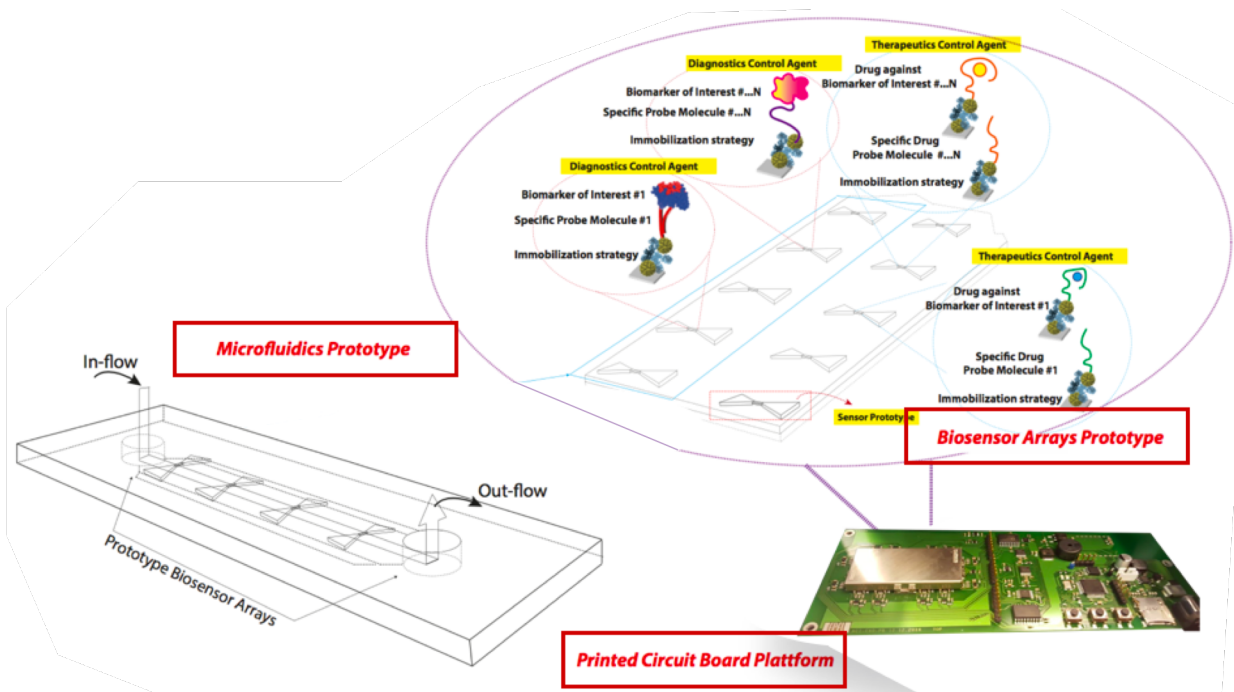


Fig. 5. Illustration of the memristive bio-sensing platform, implemented for theranostics in combination with specially designed microfluidic circuit.

performance of the nanodevices at the different antigen steps, demonstrates a decreasing behavior only for the nanodevices functionalized with the positive control antibody while the voltage gap for the other nanodevices remains constant at the levels of the values after the bio-functionalization. This finding brings proof of the possibility for efficient individualized bio-functionalization of different nanodevices on the same chip. Moreover, these results demonstrate the capability of the memristive biosensors to provide discrete sensing of a target molecule against other non-specific molecules through a tailored functionalization of individual devices. Therefore, this study consists a very important step towards future implementation of the memristive biosensors for the detection of a specific biomarker or drug when it is included in a cocktail of substances where other (non-specific) biomarkers or drugs are included as well. As a more extended version of this scheme, multiple biomarkers can be detected paving the way for multi-panel applications and simultaneous bio-detection of different biomarkers on the same chip. Having proven the multi-panel memristive bio-sensing paradigm, this scheme can be combined in a straight-forward way with the memristive biosensing board prototype [10] as well as with the microfluidic circuits especially designed for memristive biosensors [15], as illustrated in the complete scheme shown in Fig. 5.

IV. CONCLUSIONS

In this work, a proof-of-concept multi-panel on-single-chip detection scheme implementing memristive biosensors is for the first time presented and validated. First, specially designed memristive sensing chips offering disposable sensing modules by integrating memristive wires and extension metal electrodes are validated for successfully providing the electrical

characteristics of the devices before and after the bio-functionalization and the target uptake steps. Most importantly, this novel sensing paradigm demonstrates successful implementation of the memristive biosensors in a multi-panel sensing scheme in both labeled and label-free sensing. The system presented in this work paves the way for advanced bio-detection with memristive biosensors, allowing individualized detection of specific targets in complex matrixes in the presence of non-specific molecules or in even more complex schemes i.e. cells, tumor extracts, holding also great promise for fast and ultrasensitive sensing in theranostics.

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