Nano-Fabricated Memristive Biosensors for Biomedical Applications with Liquid and Dried Samples

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Abstract— **Nanowire based platforms are widely reported for sensing applications and for their potential in the bio-detection field. In the present work, memristive nanowire devices are implemented for label-free sensing in liquid samples as well as in dried samples via leveraging the modification of the hysteresis in the devices electrical response as a consequence of the surface modification. First, pH sensing in liquid conditions is demonstrated. In addition, the memristive devices are bio-functionalized using antibodies for Prostate Specific Antigen (PSA) as case of study. In this way, the nanowires are converted to memristive biosensors paving the way for future molecular diagnostics applications in general, and for detection of prostate cancer disease in particular.**

I. Introduction

Nanowire based devices, are reported for high sensitivity and applications in biosensing area, enabling direct electrical detection of biomolecules and fast, low-cost analysis of biological processes [1-3]. Compared to planar Ion-Sensitive Field-Effect Transistor (ISFET) systems, nanowires possess a large surface-to-volume ratio and therefore their electrical properties are strongly influenced by even minor perturbations [4, 5]. With one dimension much closer to the typical size of biological systems, and taking also into consideration the tunable electron transport properties that the nanowire exhibit, the interactions at the nanowire surface are likely to affect the "bulk" of the semiconductor [6]. For all these reasons, an increasing interest in the field of chemical and biological detection is expressed, with nanowire devices playing pivotal role as sensor platforms. Pronounced hysteresis in electrical characteristics is a unique and important signature of memory effects in electronic-based devices [7]. The theory for a broad range of systems showing this memory effect was developed initially by Chua [8] and more recently by Strukov et al. [9]. These memristive systems are passive systems that possess memory capability as they can maintain their specific state in the absence of an applied bias. This effect is observed at the nano-scale level where the current versus voltage characteristics appear as hysteresis loops. Such systems have been suggested for implementation in a wide variety of applications like for Resistive RAM (ReRAM) memories [10], Generic Memristive Structure (GMS) for 3-D Field Programmable and Gate Array (FPGA) applications [11] and artificial synapses [12] since they enable new possibilities for computation and non-volatile memory storage. Moreover nanofabricated devices, exhibiting memristive electrical response are implemented for bio-detection purposes leveraging the hysteresis modification after bio-functionalization with antibodies and the masking contribution to this effect introduced by the antigen uptake [13]. In the present study, memristive nanowire devices are suggested as a platform for the detection of charged chemical and biological species in liquid and in dry conditions. More specifically, pH sensing is first presented as a basic demonstration of the memristive biosensor sensitivity to surface phenomena in liquid conditions and then the potential of the platform in the bio-medical field is discussed through the device electrical characterization in dry conditions after the bio-functionalization with anti-free-PSA antibodies for future application in prostate cancer diagnostics as a case of study.

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II. Materials and Methods

A. Memristive Devices

The Memristive Devices are fabricated through a top-down fabrication process, using commercially available (100) oriented Silicon-On-Insulator wafer with low boron concentration (NA≈ 10^{15} atoms/cm^3). The nanostructures are defined using e-beam lithography, etched through a Deep Reactive Ion Etching Process (DRIE) of crystalline silicon and anchored between two NiSi junctions that form electronic contacts for the characterization. The electrical response of those devices indicates a hysteresis loop at zero voltage.

B. Chemicals

Solutions with different pH are prepared and used for the experiments. Initially, freshly prepared Phosphate Buffered Saline (PBS) (solution of pH 7.4 filtered through a syringe filter with 0.22 μm pore size and left in ultrasonic bath for 30 min for degasing) is adjusted from pH 7.4 with insertion of 1M HCl till reaching pH 4.4. In parallel, PBS solution is similarly adjusted with insertion of 1M NaOH from pH 7.4 to pH 10.3. Five solutions of intermediate values of pH are prepared by combining the starting solution to move towards either bigger or smaller values by adding small volumes of basic or acidic solutes. The pH control is performed by means of a pH-meter (pH meter, Mettler-Toledo analytical instruments). Seven pH solutions are prepared, exhibiting an average conductivity of 29.5 mS/cm (Conductivity meter, Mettler-Toledo analytical instruments). The antibody is a polyclonal rabbit anti-human PSA (Dako, A0562) in PBS (pH 7.4) against PSA-IgM complex (Xeptagen Spa).

C. Experimental procedure

Memristive silicon nanowire devices of mean width of 90 nm and length 980 nm (Fig. 1) are implemented for

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measurements with different pH buffer solutions in the range of [4:10]. A cyclic type of measurements is followed. The acquisition of the electrical characteristics starts with a solution of pH 4 then the pH-value is stepwisely varying to a maximum of pH 10 followed by a stepwisely decrease of the pH to the initial minimum value. All pH solutions are freshly prepared as described in Section II.B and the pH is also checked with a pH-meter just before use. The measurements are performed in liquid conditions and at room temperature. The devices are exposed to the fluid using 20 μL of solution.

For the bio-functionalization, the devices are first incubated in a fresh piranha solution (H_2O_2: H_2SO_4 in ratio 3:1) for 20 min in order to clean the sample surfaces from any organic residues as well as to generate surface hydroxyl-terminating groups to enable chemical attachment of the biomolecules. Subsequently, the substrates are functionalized using direct adsorption strategy by exposure to antibody solution. The nanostructures are incubated overnight at room temperature in 250 μg/mL antibody solution. The antibody solution is deposited on the surface of the substrate directly to the area of interest. The bio-functionalization procedure is further enabled by the devices' surface roughness that serves by increasing the potentially available binding area for the biomolecule adsorption. After the incubation, the substrate is gently washed three times for 5 min with PBS, and then gently dried with Nitrogen (N_2) flow before the measurements. All measurements are performed immediately after the bio-functionalization process is completed, for dry samples and in controlled environmental humidity. Current to voltage characteristics are acquired in the semi-logarithmic scale using a Keithley 4200SCS in a two terminal configuration with Source Measurement Unit (SMU), by double sweeping the source to drain voltage between -0.5 and +0.5 V with a step of 0.05 V for the pH sensing and between -2.4 V to +2.4 V with a step of 0.2 V for the bio-detection, at a fixed, 0 V back-gate potential. These measurements allow the observation of the changing hysteresis properties of the memristive sensors as a function of the ion concentration. The hysteresis is studied in terms of voltage gap calculated between the forward and backward current minima of the Ids–Vds curves. In parallel impedance data is acquired using Ivium technologies (CompactStat mobile electrochemistry instrument model) in the frequency range of [1-1000Hz], amplitude of 5 mV in a constant potential of 0.5 V. The solution exchange is performed by the removal of the existent fluid followed by cleaning of the area with deionized water before the new pH solution is introduced.

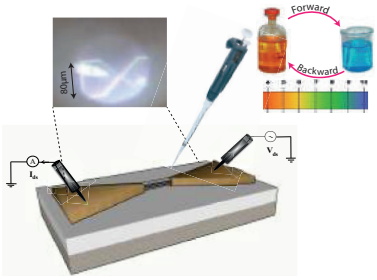

Figure 1. Schematic illustrating the experimental set-up. Memristive nanowire devices of 90 nm mean width and 980 nm of length are anchored between two pads of maximum dimension of 80μm. The insect figure demonstrates a pads-probes configuration view through optical microscope just before the electrical acquisition.

This process is performed without disturbing the measurement set-up, by using clean pipettes for the removal and the addition of the various fluids in order to achieve consistency and precision in the measurements. In addition, for reasons of consistency, AC and DC analysis are subsequently performed for each nanodevice, by properly exchanging and adjusting only the wire connections for Keithley and Ivium measurement tools respectively, without disturbing the probe-chip configuration.

III. RESULTS AND DISCUSSION

A. pH sensing

To investigate the pH sensitivity of the memristive nanowire devices, their output DC and AC electrical characteristics are obtained for different pH buffer solutions. Before bare nanowire devices the electrical characteristics demonstrate an hysteresis loop occurring at zero voltage [13]. However, this hysteresis is modified by contacting the sensor with an electrolytic medium and the current minima for the forward and backward voltage sweeps appear shifted from zero to different voltage values, introducing a voltage gap in the semi-logarithmic current to voltage characteristics. In Fig. 2 the mean behavior of the voltage gap as function of pH is presented. In particular, each point represents the mean of the voltage gaps calculated from the hysteretic curves acquired from six nanofabricated devices. The error bars in the calculation of the voltage gap calibration curve stand for the standard deviation and are highly dependent from the nanofabrication process and especially to the DRIE that results in non-homogenous structures. All sensors respond in a consistent manner regarding the average voltage gap obtained by the DC electrical characterization. Varying buffer solutions of pH produces a systematic response of the voltage gap reporting an increase of the hysteresis with increasing the pH for the forward way and vise versa for the backward regime. From pH 5 to pH 10 the voltage gap response appears linear. However, for pH values lower than pH 5, the change of the hysteresis is found to be smaller, indicating a limited buffer capacity of the surface and a leveling of the pH sensitivity. Interactions at the sensor surface, where also native oxide is formed due to the exposure to ambient oxygen, can lead to a change in the net balance of the surface charges distribution and the surface potential due to positive protonated, neutral, and negative de-protonated groups. In pH-sensitivity experiments, two different types of hysteresis might occur. The first one is an electrical hysteresis, which originates from the electrical polarization of fixed charges within the device or at the Si/native oxide interface region as well. In the memristive structures, such as the platform hereby suggested, the initial hysteresis is attributed to memory effects depending on the rearrangement of charge carriers at the nanoscale due to external perturbations like for example applied bias [9]. Nevertheless, the hysteresis modification with the introduction and variation of the voltage gap upon exposure to ionic solutions is related to the concentrations of ions "released" from the starting solution and charging the device surface. Consequently, the surface pH modification leads to an additional hysteresis (that is expressed as the modification of the initial inherent hysteresis of the nanodevice) in the electrical characteristics. A second type of hysteresis in pH-value measurements might originate from incomplete

changes of pH-solutions, especially in continuous measurements when measuring during forward and backward pH-value steps. This is mainly influenced by the geometry of the liquid cell, the method of solution exchange, and surface effects at the liquid-solid interface. Overall, it can be concluded that both electrical hysteresis and chemical hysteresis simultaneously occur. In order to further test the performance of the system, impedance measurements are performed on the same nanofabricated devices and are compared for the different pH values. The results are shown in Fig. 3 where the average value of the charge transfer resistance shift (%) exhibited by the nanodevices as a function of pH is presented. More specifically, each point represents the mean value of the changes in impedance signals calculated using the starting pH 4.4 as reference. The error bars in the calculation of the voltage gap calibration curve stand for the mean square standard deviation and are highly dependent from the top-down nanofabrication process that result in non-identical nanofabricated structures also introducing surface roughness. The measurements show a general trend where the charge transfer resistance shift occurs for higher values with increasing pH a fact that can also clearly be seen in the Nyquist plots (Fig. 3 inset) where the nanodevices impedance moves towards higher values upon increasing pH. The hysteresis originating from the incomplete changes of pH-solutions, occurring in the case of forward and backward pH-value steps is also evident since forward and backward results occur at slightly different values. In addition, the memristive nature of the nanofabricated devices is clearly demonstrated through the form of the Nyquist plot curves that is a further proof of a non-purely resistive element since memory phenomena are a particular characteristic of the nanofabricated device itself. Typically, pH sensitivity of the surface degrades over the experimental procedure due to surface contamination. This effect is even more pronounced when measuring in buffer in a forward and backward pH-value step way. In addition, the Debye length is introduced when performing measurements in liquid conditions, masking the sensing effects. The memristive nanowire sensors are implemented for pH measurements [14] as well as for bio-sensing [13] in dry conditions where the sample is thoroughly dried after the exposure to the target reagent. However, a liquid-like thin film is formed at the nanowire surface due to the ambient humidity.

B. Protein sensing

Having hereby demonstrated the memristive nanowire platform for pH sensing, the nanodevices are as well implemented for the bio-detection of charged proteins, that have an extended size and charge distribution compared to ions. To this end, the electrical response of the memristive nanowire sensors with respect to the binding of charged proteins is demonstrated through the hysteresis modification method. In order to implement biosensing, the memristive nanowire devices are converted to memristive biosensors by surface bio-modification with protein of interest. The presence of charged residues introduced by biological molecules on the device surface act by creating an electrical field surrounding the channel of the memristive device, resulting in an effect equivalent to that obtained in case of nanostructures fabricated with an all-around silicon gate [13].

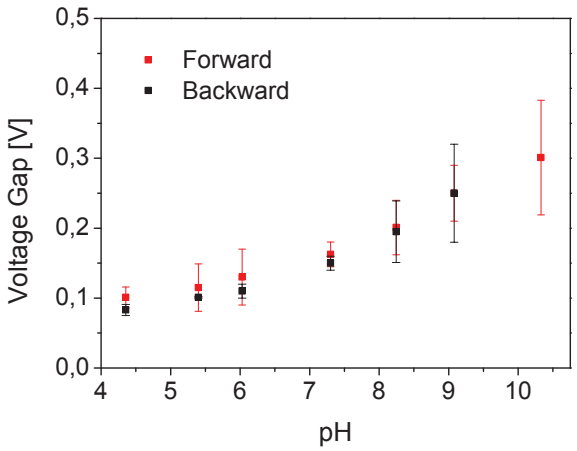

Figure 2. Average hysteresis modification (Voltage Gap) exhibited by the memrsitive nanofabricated structures, obtained through DC electrical analysis in liquid with respect to pH.

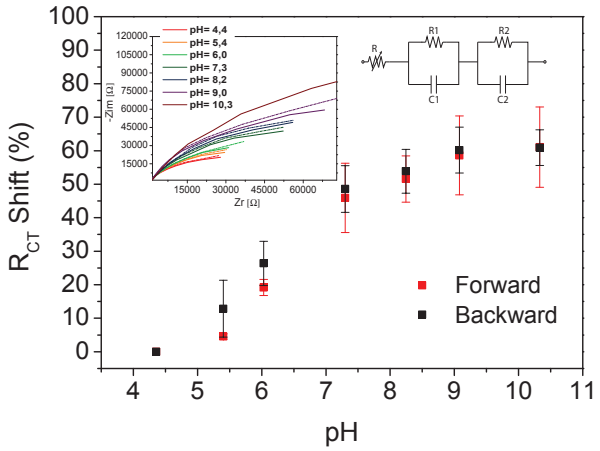

Figure 3. Average Charge transfer resistance shift (%) exhibited by the memristive nanofabricated structures, obtained through AC electrical analysis in liquid with respect to pH. The inset figure demonstrates the Nyquist plot curves for each pH value.

The virtual all-around gate effect due to the biomolecules on the nanodevice contributes to the particular conductivity of these structures, a modification of the hysteresis, introducing voltage difference in the forward and backward signals at the semi-logarithmic electrical characteristics and thus, leading to a label-free bio-detection method, which includes the presence of a voltage shift in the electrical characteristics (Fig.4 left inset). An increasing average hysteresis modification (Voltage Gap) exhibited by the memristive, bio-modified, nanofabricated structures, with increasing the relative humidity percentage (rH%) originating from the ambient atmosphere is reported in Fig.4. The environmental humidity plays a very important role influencing the memristive signals. Furthermore, the Voltage Gap response is presented with respect to the concentration of antigen uptaken on the bio-sensor (Fig.4 right inset). The results shown are obtained in rH 25% for the binding pair Polyclonal Rabbit anti-human PSA − PSA-IgM. The change in voltage gap by the increasing relative humidity demonstrates the role of the thin water molecule layer on the nanodevice surface and further enhances the mechanism of sensing.

The introduction of oppositely charged substances brings a masking effect to the already present Voltage gap [13]. Namely, the antigen uptake brings an opposite contribution to the effect introduced by the antibodies during the bio-functionalization resulting to a narrowing voltage gap with respect to increasing antigen concentration uptake (Fig 4 right inset).

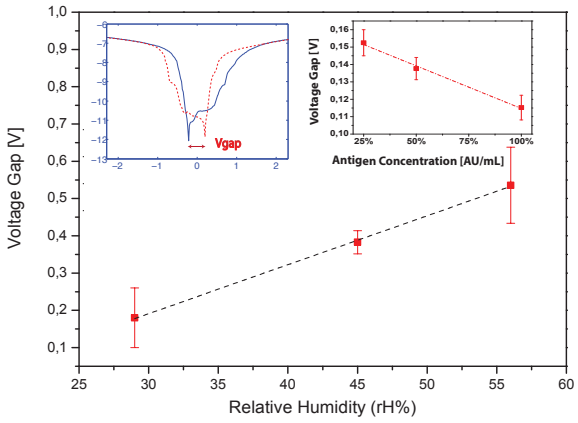

Figure 4. Voltage Gap modification obtained through DC electrical analysis in dry with respect to relative Humidity percentage (rH%). Representative electrical characteristic for rH 45% for forward (red) and backward (blue) regimes (inset figure left) and Voltage Gap response for Polyclonal Rabbit anti-human PSA − PSA-IgM Antibody-Biomarker binding pair (inset figure right).

CONCLUSION

In the present work memristive silicon nanowire devices are implemented for pH sensing in liquid through both DC and AC analysis. The nanodevices show the possibility of sensing pH variations by the memristive effect. The data acquired while measuring in liquid conditions are in good agreement with those applied on dried samples. This demonstrates the ability of the memristive sensors for performance in both liquid and dry conditions. In addition the memristive nanowire devices are further converted to memristive biosensors by surface functionalization with antibody-probes against PSA. This finally demonstrates the potential of these memristive sensors for applications in bio-sensing and medical applications.

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REFERENCES

[1] N. Elfstrom, R. Juhasz, I. Sychugov, T. Engfeldt, A. Karlstrom, J. Linnros, Surface Charge Sensitivity of Silicon Nanowires: Size Dependence, *Nano Lett.* 7(9):2608-2612, 2007.

[2] F. Ishikawa, H.K. Chang, M. Curreli, H.I. Liao, A. Olson, P.C. Chen, R. Zhang, R. Roberts, R. Sun, R. Cote, et al. Label-Free, Electrical Detection of the SARS Virus N-Protein with Nanowire Biosensors Utilizing Antibody Mimics as Capture Probes, *ACS Nano* 3(5):1219–1224, 2009.

[3] F. Patolsky, G. Zheng, C.M. Lieber, Nanowire sensors for medicine and the life sciences, *Nature Biotechnology*, 1(1): 51–65, 2006.

[4] F. Patolsky, G. Zheng, C.M. Lieber, Fabrication of silicon nanowire devices for ultrasensitive, label-free, real-time detection of biological and chemical species, *Nature Protocols*, 1: 1711–1724, 2006.

[5] X.T. Zhou, J.Q. Hu, C.P. Li, D.D.D. Ma, S.T. Lee, Silicon nanowires as chemical sensors, *Chem. Phys. Lett.* 369: 220–224, 2003.

[6] Y. Cui, Q. Wei, H. Park, C. M. Lieber, Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species, *Science*, 293(5533):1289-92, 2001.

[7] J. Wua, R. McCreery, Solid-State Electrochemistry in Molecule/TiO_2 Molecular Heterojunctions as the Basis of the TiO_2 “Memristor”, *J. Electrochem. Soc.* 156 (1): 29-37, 2009.

[8] L. Chua, Memristor-The missing circuit element, *IEEE Trans. Circuit Theory*, 18(5): 507 – 519, 1971.

[9] D. Strukov, G. Snider, D. Stewart, S.Williams, The missing memristor found, *Nature*, 453: 80-83, 2008.

[10] D. Sacchetto, P.E. Gaillardon, M. Zervas, S. Carrara, G. De Micheli, Y. Leblebici, Applications of Multi-Terminal Memristive Devices: A Review, *IEEE Circuits and Systems*, 13(2): 23-41, 2013.

[11] P.E. Gaillardon, D. Sacchetto, S. Bobba, Y. Leblebici, GMS: Generic Memristive Structure for Non-Volatile FPGAs, IFIP/IEEE International Conference on Very Large Scale Integration (VLSI-SoC), Santa Cruz, California, USA, 2012.

[12] G. Indiveri, B. Linares-Barranco, R. Legenstein, G. Deligeorgis, T. Prodromakis, Integration of nanoscale memristor synapses in neuromorphic computing Architectures, *Nanotechnology* 24:384010, 2013.

[13] S. Carrara, D. Sacchetto, M.A. Doucey, C. Baj-Rossi, G. De Micheli, Y. Leblebici,Memristive-biosensors: A new detection method by using nanofabricated memristors, *Sensor Actuat. B-Chem*. 171-172: 449-457, 2012.

[14] F. Puppo, M. Di Ventra, G. De Micheli, S. Carrara, Memristive sensors for pH measure in dry conditions. *Surf. Sci.* 624: 76–79, 2014.