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Preliminary Study of a Low-Cost Point-of-Care Testing System Using Screen-Printed Biosensors

for Early Biomarkers Detection Related to Alzheimer Disease

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Abstract — **Among neurodegenerative diseases, Alzheimer Disease (AD) represents one of the most widespread pathologies, for which an early diagnosis is still missing. A peculiar expression of an altered conformational isoform of p53 protein was reported to be a potential biomarker able to distinguish AD subjects from healthy population, quantifiable using a blood-based enzyme-linked immunosorbent assay (ELISA). In order to overcome ELISA limitations, related to reliability and to improve sensitivity, this study aimed to realize a low cost highly sensitive portable point-of-care (PoC) testing system based on screen printed electrochemical sensors (SPES). The development of the platform specifically included both the design of the sensing probe and of the electronic circuit devoted to the conditioning and acquisition of the transduced electric signal. In particular, silver, carbon and silver-silver chloride were selected respectively to realize conductive tracks, working and counter electrodes, reference electrode in a three-electrodes configuration focusing on Anodic Stripping Voltammetry (ASV). The conditioning circuit was designed following the scheme for a common potentiostat, and produced as a Printed Circuit Board (PCB). Initial testing of the circuit were performed recording changes in the conductivity of NaCl solution and quantifying electrodes coating with antibodies using Electrochemical Impedance Spectroscopy (EIS) principle. Preliminary results obtained with saline solution, showed the ability of the circuit to give the best response corresponding to low changes in NaCl concentration (sensitivity 0.2 mA/(mg/ml)), suggesting a good sensitivity of the platform. Results from EIS showed the ability of the circuit to discriminate between different concentrations of antibodies coatings (sensitivity 80 µA/µg). The study is on-going and after a proper calibration, the circuit is intended to be optimized to quantify unknown concentration of unfolded p53 in samples of peripheral blood of AD patients, compared results with the one from ELISA analysis, aiming to realize a low cost, easy to use and highly precise platform.**

Keywords— screen printing; electrochemical biosensors; biomarkers; Alzheimer disease; point of care testing

I. INTRODUCTION

Among neurodegenerative diseases, Alzheimer Disease (AD) represents one of the most investigated and serious pathologies, for which an early reliable diagnosis is still missing. However, AD presents a long pre-symptomatic period, that could last for 20 years, and which is characterized by biochemical and molecular events that can be used to foresee the beginning of the disease itself. Therefore, one of the most pervasive challenges of the research in medical diagnosis is related to the ability to detect this pathology in its earliest development [1], [2], thanks to reliable identification of specific biomarkers, or biological markers (e.g. proteins). Biomarkers represent in fact an indicator of the biological status, which can give useful information concerning physiological or pathological conditions and during the application of different medical treatments. Concerning AD diagnosis, recent studies specifically reported how the presence of a particular altered conformation of a specific protein (p53) could be able to discriminate subjects affected by AD from healthy population [3]–[5]. In this perspective, clinicians require technologies able to identify, quickly and with a high sensitivity, specific biological biomarkers related to AD disease, overcoming ELISA issues in term of time and cost effectiveness, volume of sample required, reliability and possibility of quantification. In the last decades, new rapid, low cost and easily accessible methodologies and technologies have been increasingly investigated, supported by the interest toward customized and personalized medicine and toward rapid and home-accessible diagnostic systems [6]–[8]. The idea of reliably quantifying the presence of defined biomarkers in the early stages of a specific disease appears to be really up-and-coming. Indeed, biosensors integrated in lab-on-a-chip (LoC) devices represent promising methods to reduce time, cost and quantity of sample needed to perform the analysis [2], [7], [8]. Thanks to their ability to be functionalized and customized for the detection of different analytes (e.g. DNA, proteins) electrochemical biosensors, represent the ideal starting point to realize complete testing platforms. By integrating sensors with proper conditioning and acquisition circuit is in fact possible to realize portable and self-standing devices, fundamental for point-of-care (PoC) applications [6].

Printed electronics represents a successful approach to realize low cost and sensitive biosensors. In particular, literature reports different studies based on screen printing

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(also defined thick film printing) to realize electrochemical sensors for the sensitive and specific quantification of different proteins [9]–[13]. By implementing biosensors, different methods can be used to detect and quantify specific proteins. The most widely used methodology for immunosensors is based on the electrode surface functionalization, very similar to those used in the multi-wells standard enzyme-linked immunosorbent assay (ELISA) [13]. A label-free method is represented from Electrochemical Impedance Spectroscopy (EIS), which measures the changes of impedance deriving from a different electrons exchange between the functionalized surface of the electrode and a conductive solution (usually potassium ferricyanide, $K_3[Fe(CN)_6]$), depending on the concentration of the recognized proteins [7], [9], [10]. For this specific technique, usually a 2–electrode conformation - including a working (WE) and a reference electrode (RE) - can be used. Besides EIS, voltammetry can be also implemented as measuring method, with a 2- or 3-electrodes conformation depending on the required level of precision and sensitivity; a 3-electrode conformation - including WE, RE and a counter electrode (CE), used to measure the current – usually allows for optimal measurement within this kind of applications [14], [15]. This method is very fundamental in PoC implementation since it can be performed both with the sensor completely immersed in a buffer solution [10] or by placing a drop of solution which cover the three electrodes, thus requiring small volume of samples [14]. Further, Anodic Stripping Voltammetry (ASV) represents a very sensitive kind of voltammetry technique able to detect both proteins [14] or DNA sequences [16], with limit of detection in the order of ng/ml, by using – for instance - the action of specific catalyzing enzymes. In this way, the concentration of proteins can be estimated as proportional to the peak of current measured during a stripping step, varying the potential between WE and RE. To further improve the specificity and the sensitivity of this technology, limits of detection lower than 100 pg/ml have been achieved thanks to the integration of the SPES with nanostructured materials. Gold nanoparticles for example or carbon nanotubes or a combination of the two has been used to modify the surface of the working electrode allowed to better recognize antigens and DNA sequences [14], [16]–[18].

Focusing on the quantification of a specific biomarker for the early detection of AD, the main objective of this work addressed the preliminary study of a low-cost portable point-of-care testing platform for the detection and sensitive quantification of the unfolded p53 protein. The study specifically reported the design of the sensing probe, with particular attention to the choice of the materials and geometry, and of the electronic circuit devoted to the conditioning and acquisition of the electric signal. In particular, the electrodes were designed to be easily implemented by means of screen printing methodology. Considering the whole testing workflow and without losing the possibility of generalization, preliminary results were obtained on controlled concentrations of electrolytic solution.

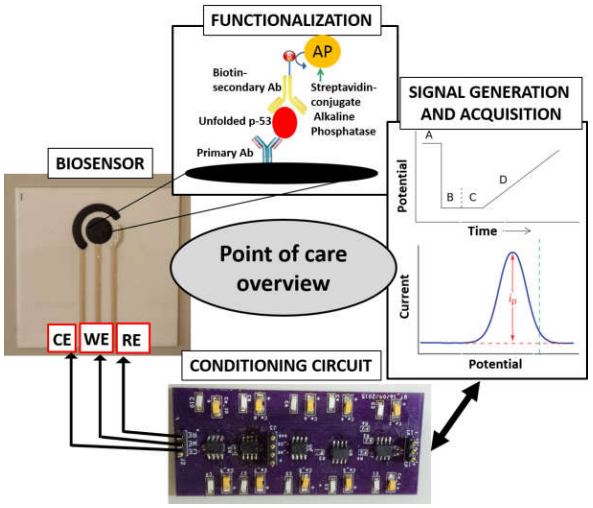

Fig. 1. Overview of the Point of Care Platform

II. System Description, Materials and Methods

The purposed point of care is composed by three main parts: the screen printed sensing probe, which will be the heart of our work, functionalized using specific antibodies for our biomarkers; the PCB for biosensor conditioning, highly precise and sensitive, in order to register small changes in ionic current during EIS and ASV protocols; and finally the electronic instrumentation for the generation and acquisition of the signal (Fig. 1).

A. Sensors Design and Production

Sensor layout was designed using QCAD software (QCAD.org). Each layer, corresponding to a different conducting material, was separately designed, in order to produce the masks required to screen print - layer by layer - the final structure of the sensor. Conductive tracks were designed with particular care using a resolution compatible with the printing performance of the adopted screen printer (A2 Model, Baccini srl, Italy). The optimized geometry - with a WE of 8.0 mm of diameter - was printed on a lucid sheet by means of inkjet printing, thus to allow the realization by UV photolithography of a 40 µm thick blocking stencil, the mask required for the screen printing process. Conductive pastes were purchased from ECM – Engineered Conductive Materials (Engineered Material System Inc.). Specifically, a silver ink (CI 1001, electrical resistance < 0.015 ohms/square, viscosity 14 Pa s @ 30° C) was selected to realize the conductive tracks. Carbon ink was selected to realize the working and the counter electrode of the biosensor, because of carbon biocompatibility and efficiency in signal transduction. Finally, a silver-silver chloride ink (CI 4002, electrical resistance < 0.050 ohms/square, viscosity 5 Pa s @ 30° C) thanks to its inert chemical composition, was selected to realize the reference electrode. Using a screen with 250 meshes with apertures forming angles of 45 degrees, the three layers were consequently printed on a 0.4 mm thick alumina substrate: first silver, then carbon and finally silver-silver chloride. Before proceeding with the next layer, each of the layers was cured in an oven, specifically

silver inks for 10 min at 110 °C and carbon ink 5 min at 130 °C. After the printing process was completed, in order to allow a better conduction of the signal, the conductive tracks were isolated using a conductive spray specifically adopted in printed electronics, leaving the terminal part of the tracks free for the connection with the conditioning circuit (Fig. 2).

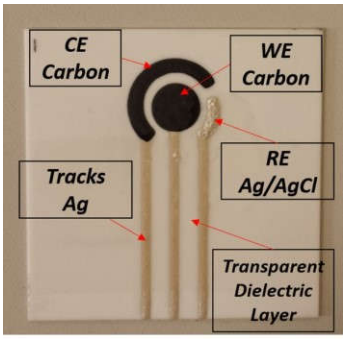

Fig. 2. Final SPES layout.

B. *Circuit Design and Production*

Parallelly to the realization of the electrochemical sensor, the conditioning and acquisition circuit was designed in order to allow the production of a complete PoC testing platform. The design of the circuit was in particular performed by following the scheme of common potentiostats (Fig. 3), in which a varying potential is applied to the WE with respect to the RE (which is analogous to system ground) and, thanks to the electrons movement generated by the effect of the applied electromotive force in the sample, the generated current is then detected by the CE.

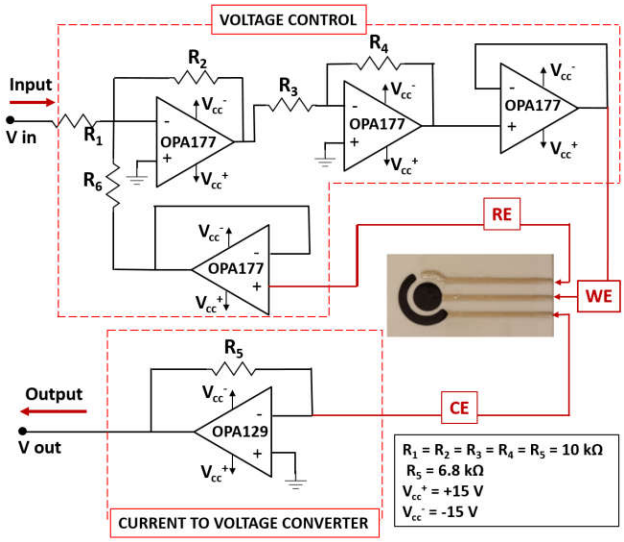

Fig. 3. Schematic of the potentiostat unsed as conditioning circuit

The electronic schematic was realized with OrCAD software (OrCAD©- Cadence Design Systems-San Jose, CA) whereas the design for the Printed Circuit Board (PCB) was prepared using OrCAD Layout Plus tool (OrCAD ©-Cadence Design Systems-San Jose, CA). Operational amplifiers were carefully selected depending on the specific function they had to perform in the circuit. In order to guarantee a high accuracy in the control of the input voltage to the sensor, precision bipolar amplifiers were selected (OPA177GS from Texas Instrument (TI)), with very low offset voltage, drift, and low noise. In order to allow low current measurements with high precision and low bias, the current-to-voltage converter was realized using an ultra-low bias current monolithic operational amplifier (OPA129 from TI). After all the SMD components were soldered (Fig. 4), the board was inserted in a metallic box to avoid noises on the signal recording and to improve the sensor sensitivity and precision.

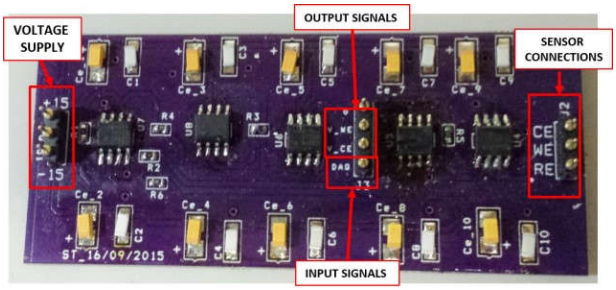

Fig. 4. Final PCB of the conditioning circuit

C. *Preliminary analyses*

Before designing and producing the platform itself, the adhesion of different concentration of antibodies solutions was evaluated using carbon electrodes (of the same dimensions of the WE in the final SPES layout) and silver chloride references, in order to optimize the best concentration for an efficient functionalization of the biosensor. Two different techniques have been specifically chosen for the analysis: an electronic method, EIS, and an optical one, evaluating the light intensity thanks to fluorescent labels on the secondary antibody. EIS measurements were performed measuring the impedance between WE and RE on a wide range of frequencies (200 Hz –200 kHz) in an electrolytic solution of 5 mM $K_3[Fe(CN)_6]$) in 1M KCl. Impedance measurements were specifically performed using an impedance analyzer (HP4194A, Hewlett-Packard, USA), compared with the fluorescence measurements registered using a light intensity quantifier (Odyssey® Fc Dual-ModeImaging System from LI-COR Biosciences).

D. *Calibration and measurements*

After optimizing the design, the platform was assessed following two different protocols: 1) using a saline solution, with different concentrations of NaCl; 2) functionalizing the WE with primary antibodies and applying EIS principle in presence of $K_3[Fe(CN)_6]$ solution, measuring output current as an indicator of the impedance change of the system. Experiments were always performed in triplicate. All graphical and tabulated data were usually reported as mean ± mean standard error.

1) NaCl solution measurements

The first test of the circuit was performed using a saline solution, varying the concentration of NaCl in order to change the conductivity of the solution, and evaluating the ability of the circuit to quantify these variations as changes in the current peak flowing between WE and CE.

In the first test, the concentrations of the evaluated solutions were specifically 0.00, 15.00, 22.50, 30.00, 37.50 and 45.00 mg/ml. After that, a second set of concentrations was tested, to assess the linearity of the circuit response for lower values of concentrations; in particular, the test considered 0.00, 1.00, 2.20, 4.00, 5.50, 6.00, 10.40 mg/ml. Finally, the circuit was evaluated with concentrations lower than 1.0 mg/ml, in particular: 0.00, 0.44, 0.66, 0.88, 1.00 mg/ml. In each experiment, drops of 2 ml of saline solution were released on WE, CE and RE, ensuring that the drop stayed correctly in place by using a mask applied on top of the sensor.

For this analysis, the input signal was considered as a triangular wave, with amplitude 300 mV and frequency 40 mHz, obtained using a pulse generator (HP8116A pulse/function generator 50 MHz). The signal was then acquired using an oscilloscope (Tektronik TDS 1001B – two channel digital storage oscilloscope 40 MHz, 500 MS/s).

2) Antibodies coating quantification using EIS

EIS principle was applied in order to measure changes of system impedance, resulting in changes in the current detected between WE and CE deriving from different concentrations of the primary antibodies released and adhered on WE surface. In particular, three antibodies concentrations were considered: 0, 4 and 8 µg/ml.

After an overnight incubation at 4 °C the measurements were performed in presence of a conductive electrolytic an solution of 5 mM K3[Fe(CN)6] in 1 M KCl. Once the functionalization was performed, a drop of 2 ml was placed in order to cover WE, RE and CE and allow current flow, and the electronic measurement were performed, giving a ramp as signal input, and recording the current between WE and CE using an oscilloscope. In particular, a first analysis was performed using triangular waves four different frequencies (40 mHz, 100 mHz, 200 mHz and 1 Hz) with an amplitude of 300 mV, and then a second one fixing the frequency to 50 mHz.

III. Results

A. Preliminary analyses

Regarding the compatibility of the materials and of the printing process with wet lab practices, alumina substrate represented the optimal solution. Thanks to the intrinsic porosity of the material, electrodes printed on this substrate did not show any variation when washed with water-based solutions during functionalization steps. On the contrary, electrodes printed on glass and polystyrene, because of their low porosity, did not show an efficient adhesion, with critical modifications during the functionalization step, compromising the uniformity of the primary antibodies coating on the WE and the effective complex formation with the secondary antibody. Among the different primary antibody concentrations evaluated on alumina (2.0, 2.6, 3.0, 4.0, 4.8, 6.0, 8.0, 10.0 µg/ml), 8.0 µg/ml was identified as the optimal one to achieve a homogeneous coating of the WE. The fluorescence signal showed a value of intensity proportional to the concentration of primary antibody coated in the range between 2 and 6 µg/ml (Fig. 5).

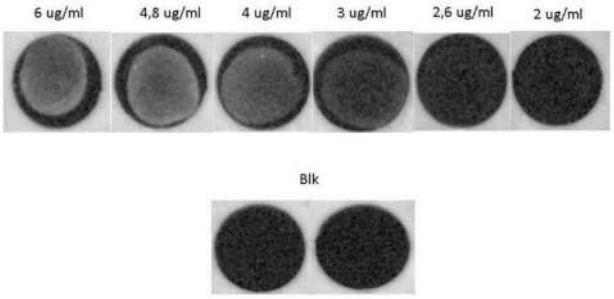

Fig. 5. Image obtained from the optical analysis of WE coated with different primary antibody concentrations.

Impedance measurements performed on the same electrodes, showed results in agreement with what previously evaluated with optical analysis. More specifically, the linearity observed for concentration of 0.0, 6.0, 8.0 µg/ml, could be observed with EIS as well. The impedance module measured with electrodes coated with 8.0 µg/ml primary antibody solution resulted to be superior in all the frequencies range evaluated, compared with the one of blank electrodes, treated with a buffer solution (mean 15.5 ± 4.6 Ω between 2 and 20 kHz; max 30 Ω at 2 kHz and minimum of 9 Ohm at 200 kHz). The impedance module measured with electrodes treated with 6.0 µg/ml showed a trend comprised between the previous two (Fig. 6).

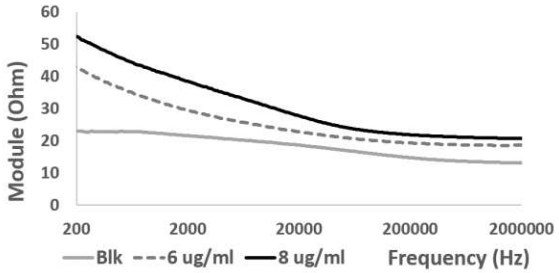

Fig. 6. Electronical measurements (EIS) of different antibody concentration coatings.

B. Calibration and measurements

1) NaCl solution measurements

Results from the evaluation of circuit response to changes in saline solution conductivity showed a linear trend for the ranges of concentrations specifically evaluated. After assessing for linearity using high concentration of NaCl, a narrower range of concentrations was also considered in order to understand if the circuit was able to recognize small changes in solution conductivity corresponding to small values of current flowing between WE and CE. Performing

the same measures with lower concentration, a particular behavior could be noticed. Two different slopes could be observed respectively for concentration lower and higher than 1.0 mg/ml. In particular, a higher sensitivity was shown for the concentration lower than 1.0 mg/ml, indicating a higher sensitivity of the sensor (0.2 mA/(mg/ml)) for small changes of conductivity and small currents, and a lower sensitivity (20 µA/(mg/ml)) for higher concentration (Fig. 7). This is probably due to the specific features of the SMD components chosen, which makes them from one side really precise and sensitive to detect small changes in ionic current, but from the other unable to detect with the same sensitivity higher conductivity variations, which brings to current which cause the circuit to saturate.

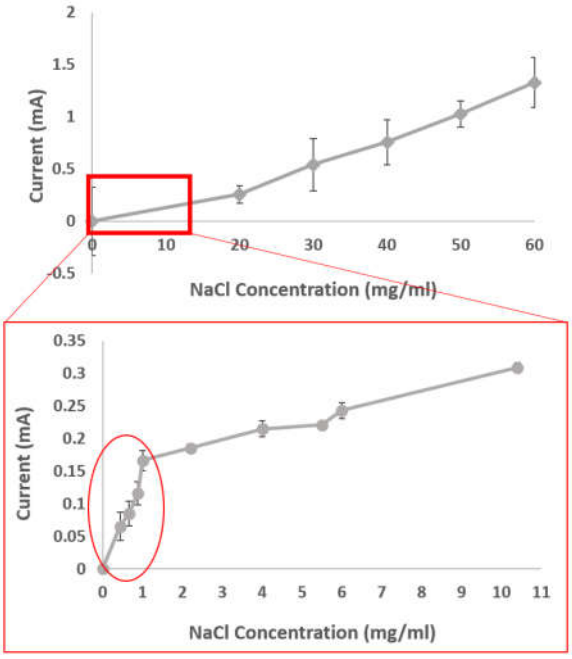

Fig. 7. Calibration of SPES with NaCl solution.

1) Antibodies quantification using EIS

Results from EIS measurements showed a proportional decreasing of the peak of current flowing between CE and WE, indicating an increased impedance of the system due to an increasing concentration of antibodies coated on WE surfaces resulting in a reduced electrons exchange between WE surface and electrolytic solution (Fig 8 and 9).

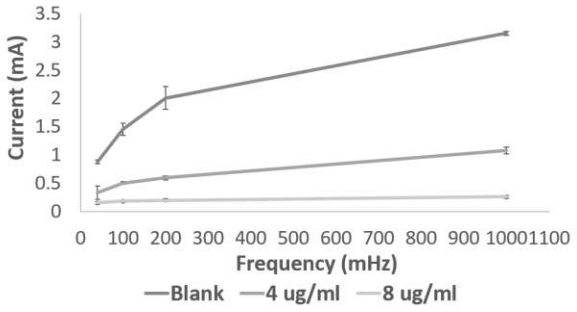

Fig. 8. Difference in CE current measured at different frequencies, evaluating with EIS different concentration primary antibodies coatings.

The same behavior was observed at all the frequencies evaluated. Increasing the concentration of antibodies coated on the WE resulted in reducing the differences of currents exchanged at different frequencies (Fig. 9). As showed in Fig. 9, the sensitivity of the sensor in detecting the change in antibodies coating concentration was of 80 µA/µg.

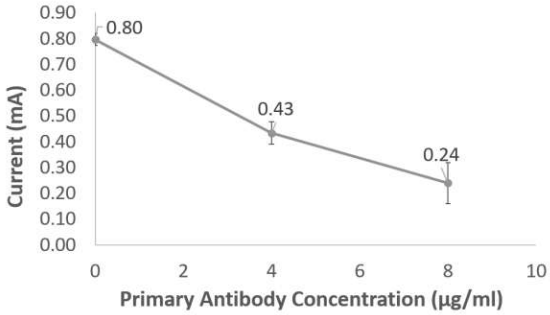

Fig. 9. Difference in CE current measured at 50mHz, evaluating with EIS different concentration primary antibodies coatings.

IV. Discussion

The complexities and the heterogeneity associated with AD, requires high precision and sensitivity in the reliable detection and quantification of specific biomarkers, able to allow an early diagnosis of the disease in the pre-symptomatic phase and to acquire additional information both from the biological and from the pathoclinical point of view [2], [19]. In order to efficiently achieve this goal, rapidity, low cost and ease of access of the methodology play an important role, in the interest toward customized medicine and toward rapid and home accessible diagnostic results [6].

In this preliminary study, the obtained results confirmed the compatibility of the screen printed materials for the functionalization with AD biomarkers and the wet lab practices. Furthermore, testing of the circuit performed both with NaCl solution and with K3[Fe(CN)6 solution, validate the ability of the designed circuit to effectively detect changes in solution conductivity and system impedance, thanks to changes in the current flowing between WE and CE. Even if the limit of detection were still higher than the ones we aimed to obtain for p53 sensitive detection [17], [18], results obtained using NaCl showed a particular behavior which suggested that the range of concentration in which the circuit showed a higher sensitivity corresponds to low changes in concentration, resulting in small ionic currents (Fig. 6). The response of the circuit showed during the EIS measurements confirmed this suggestion, showing good reproducibility, reliable responses, and a sensitivity (80 µA/µg) promising for the future developments with lower values of proteins concentrations. An improvement in term of standardization of the procedure will be required and will be our main goal: minimize the variability deriving from the production and functionalization of the biosensor, introduce an automatized measurement system and improve the point of care portability will be necessary to get to a self-standing testing platform.

V. Future outlooks and conclusion

In light of the positive results described, the activity actually going on refers to the implementation of the same protocol described in the materials and methods section using interleukin protein, of dedicated kit (DuoSet® development system for ELISA, Human CXCL8/IL-8). Different concentrations of proteins (order of ng/ml) will be recognized using both the techniques described (EIS and ASV). This phase is essential for an effective calibration of the platform, in order to proceed with the quantification of unknown concentration of unfolded p53 protein. Specifically regarding ASV, the same protocol will be adopted to quantify protein concentrations, both for interleukin and for p53 proteins. It will be characterized by the use of an immucomplex formed by a capture and a detection antibody labelled with Alkaline Phosphatase, as functionalization of the WE. In this way, through a selective chemical deposition of silver, the current flowing between WE and CE will be proportional to the amount of deposited silver, which in its turns will be proportional to the recognized protein. Before proceeding with unknown proteins concentrations, an accurate calibration of the biosensor will be performed. After the validation, the proposed methodology and the platform design will be optimized in order to be easily accessible for a routine automatized diagnosis technique in the clinical environment. From these bases, particular attention will be then addressed to increase the sensitivity of the method itself, including both the introduction of nanostructured materials for the working electrodes and proper ASV measurements. All this, with the aim to realize an innovative self-standing portable point-of-care, a low cost, easy to use and highly precise platform able to support clinicians to diagnose AD from its earliest stages.

References

[1] Z. Svobodova, M. Reza Mohamadi, B. Jankovicova, H. Esselmann, R. Verpillot, M. Otto, M. Taverna, J. Wiltfang, J.-L. Viovy, and Z. Bilkova, "Development of a magnetic immunosorbent for on-chip preconcentration of amyloid beta isoforms: Representatives of Alzheimer's disease biomarkers.," *Biomicrofluidics*, vol. 6, no. 2, pp. 24126–2412612, Jun. 2012.

[2] C. Humpel, "Identifying and validating biomarkers for Alzheimer's disease," *Trends Biotechnol.*, vol. 29, no. 1, pp. 26–32, Jan. 2011.

[3] D. Uberti, C. Lanni, T. Carsana, S. Francisconi, C. Missale, M. Racchi, S. Govoni, and M. Memo, "Identification of a mutant-like conformation of p53 in fibroblasts from sporadic Alzheimer's disease patients.," *Neurobiol. Aging*, vol. 27, no. 9, pp. 1193–1201, Sep. 2006.

[4] C. Lanni, M. Racchi, G. Mazzini, A. Ranzenigo, R. Polotti, E. Sinforiani, L. Olivari, M. Barcikowska, M. Styczynska, J. Kuznicki, A. Szybinska, S. Govoni, M. Memo, and D. Uberti, "Conformationally altered p53: a novel Alzheimer's disease marker?," *Mol. Psychiatry*, vol. 13, no. 6, pp. 641–647, Jun. 2008.

[5] L. Buizza, G. Cenini, C. Lanni, G. Ferrari-Toninelli, C. Prandelli, S. Govoni, E. Buoso, M. Racchi, M. Barcikowska, M. Styczynska, A. Szybinska, D. A. Butterfield, M. Memo, and D. Uberti, "Conformational altered p53 as an early marker of oxidative stress in Alzheimer's disease.," *PLoS One*, vol. 7, no. 1, p. e29789, 2012.

[6] A. P. Dhawan, W. J. Heetderks, M. Pavel, S. Acharya, M. Akay, A. Mairal, B. Wheeler, C. C. Dacso, T. Sunder, N. Lovell, M. Gerber, M. Shah, S. G. Senthilvel, M. D. Wang, and B. Bhargava, "Current and Future Challenges in Point-of-Care Technologies: A Paradigm-Shift in Affordable Global Healthcare With Personalized and Preventive Medicine," *Translational Engineering in Health and Medicine, IEEE Journal of*, vol. 3. pp. 1–10, 2015.

[7] D. Polese, A. Convertino, L. Maiolo, A. Ferrone, L. Pazzini, M. Marrani, F. Maita, A. Pecora, G. Fortunato, and G. Fiaschi, "Investigation on nanostructured biosensor for Biotin detection," *SENSORS, 2014 IEEE*. pp. 1627–1630, 2014.

[8] S. Feng, L. E. Roseng, and T. Dong, "Quantitative detection of Escherichia coli and measurement of urinary tract infection diagnosis possibility by use of a portable, handheld sensor," *Medical Measurements and Applications (MeMeA), 2015 IEEE International Symposium on*. pp. 586–589, 2015.

[9] M. M. S. Silva, a. C. M. S. Dias, M. T. Cordeiro, E. Marques, M. O. F. Goulart, and R. F. Dutra, "A thiophene-modified screen printed electrode for detection of dengue virus NS1 protein," *Talanta*, vol. 128, pp. 505–510, 2014.

[10] R. Elshafey, A. C. Tavares, M. Siaj, and M. Zourob, "Electrochemical impedance immunosensor based on gold nanoparticles-protein G for the detection of cancer marker epidermal growth factor receptor in human plasma and brain tissue.," *Biosens. Bioelectron.*, vol. 50, pp. 143–9, 2013.

[11] Y. H. Yun, B. K. Lee, J. S. Choi, S. Kim, B. Yoo, Y. S. Kim, K. Park, and Y. W. Cho, "A glucose sensor fabricated by piezoelectric inkjet printing of conducting polymers and bienzymes.," *Anal. Sci.*, vol. 27, no. 4, p. 375, 2011.

[12] A. M. Pernia, M. J. Prieto, I. C. Orille, J. A. Martín-Ramos, and A. Costa-Garcia, "Development of Optimized Screen-Printed Immunosensors," *Instrumentation and Measurement, IEEE Transactions on*, vol. 58, no. 7. pp. 2181–2188, 2009.

[13] R. A. S. Couto, J. L. F. C. Lima, and M. B. Quinaz, "Recent developments, characteristics and potential applications of screen-printed electrodes in pharmaceutical and biological analysis," *Talanta*, vol. 146, pp. 801–814, Jan. 2016.

[14] V. Escamilla-Gómez, D. Hernández-Santos, M. B. González-García, J. M. Pingarrón-Carrazón, and A. Costa-García, "Simultaneous detection of free and total prostate specific antigen on a screen-printed electrochemical dual sensor," *Biosens. Bioelectron.*, vol. 24, pp. 2678–2683, 2009.

[15] Y.-F. Liang, C.-Y. Huang, and B.-D. Liu, "A Voltammetry Potentiostat Design for Large Dynamic Range Current Measurement," *Intelligent Computation and Bio-Medical Instrumentation (ICBMI), 2011 International Conference on*. pp. 260–263, 2011.

[16] G. Martínez-Paredes, M. B. González-García, and A. Costa-García, "Genosensor for detection of four pneumoniae bacteria using gold nanostructured screen-printed carbon electrodes as transducers," *Sensors Actuators, B Chem.*, vol. 149, pp. 329–335, 2010.

[17] P. Kara, A. de la Escosura-Muñiz, M. Maltez-da Costa, M. Guix, M. Ozsoz, and A. Merkoçi, "Aptamers based electrochemical biosensor for protein detection using carbon nanotubes platforms," *Biosens. Bioelectron.*, vol. 26, pp. 1715–1718, 2010.

[18] B. Jeong, R. Akter, O. H. Han, and C. K. Rhee, "Increased Electrocatalyzed Performance through Dendrimer- Encapsulated Gold Nanoparticles and Carbon Nanotube-Assisted Multiple Bienzymatic Labels: Highly Sensitive Electrochemical Immunosensor for Protein Detection," 2013.

[19] L. J. Thal, K. Kantarci, E. M. Reiman, W. E. Klunk, M. W. Weiner, H. Zetterberg, D. Galasko, D. Praticò, S. Griffin, D. Schenk, and E. Siemers, "The Role of Biomarkers in Clinical Trials for Alzheimer Disease," *Alzheimer Dis. Assoc. Disord.*, vol. 20, no. 1, pp. 6–15, 2006.