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Screen-Printed Biosensors for the Early Detection of Biomarkers Related to Alzheimer Disease: Preliminary Results

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Abstract

Alzheimer disease (AD), despite representing the most common type of dementia in elderly, is still lacking reliable methodologies for early diagnosis. A potential biomarker associated to AD development has been recently identified in the open isoform of p53, redox sensitive protein, currently quantified using a specific blood-based enzyme-linked immunosorbent assay (ELISA). In order to overcome ELISA limitations (level of detection, standardization and reliability), 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 study specifically reported the design of the platform, including the sensing probe and the electronic circuit devoted to the conditioning of the electric signal. Preliminary results were obtained from circuit testing by using controlled concentrations of electrolytic solutions and from an initial calibration stage by using Anodic Stripping Voltammetry (ASV) measurements. Future works will address the quantification of unknown concentration of unfolded p53 in peripheral blood samples, thus to validate the here-presented low cost, easy to use and highly precise platform.

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Keywords: screen printing; electrochemical biosensors; biomarkers; Alzheimer disease; point of care (PoC) testing

1. Introduction

Among neurodegenerative diseases, Alzheimer disease (AD) represents one of the most investigated and serious pathology, for which an early diagnosis is still missing. The ability to diagnose the pathology at an early stage is one

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of the actual priority of biomedical research in terms of neurology and geriatrics [1]. AD biomarker technologies currently under development include in vivo brain imaging and biochemical assays in cerebrospinal fluid (CSF) or peripheral tissues. Since AD is a systemic disease with associated dysfunction in metabolic, oxidative, inflammatory, and biochemical pathways in peripheral tissues, recently new minimally invasive approaches to investigate and develop assays for the identification of peripheral AD biomarkers [2] have been established and novel ones discussed. In particular a peculiar expression of an altered conformational isoform of p53 protein was reported to be able to distinguish AD subjects from healthy population, demonstrating high values of sensitivity and specificity [3]. On this basis, the levels of p53 unfolded could represent an interesting starting point to define a reliable blood-based ELISA performed with a specific antibody. Even if ELISA assay indeed represents the gold standard technique used for the detection and quantification of this biomarker, some limitations are related to its diffusion: high costs of the assay implementation, high operator dependence, lack of standardization and impossibility to lower the limit of detection.

In this perspective, new rapid, low cost and easily accessible methodologies and technologies have been increasingly investigated, supported by the interest towards both customized and personalized medicine, and rapid and home-accessible diagnostic systems [4], [5]. 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 [1], [5]. 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 a proper conditioning and acquisition electronic circuit, it is in fact possible to realize portable and self-standing devices, fundamental for point-of-care (PoC) applications [4]. In light of this, the main objective of this work addressed the preliminary study of a low-cost portable point-of-care testing platform, intended to improve the detection and sensitive quantification of unfolded p53 protein as a new putative AD biomarker, yet without losing the possibility to extend the proposed solution to different analytes.

2. Materials and methods

2.1. Point of care design and production

The development of the platform specifically included both the design of the screen-printed sensing probe and of the electronic circuit devoted to the conditioning and acquisition of the transduced electric signal (Fig.1). After designing the sensor geometry and producing the proper mask to perform the printing process, silver, carbon and silver-silver chloride were selected respectively to realize conductive tracks, working (WE) and counter electrode (CE), reference electrode (RE), with a three-electrodes configuration focusing on ASV measurements.

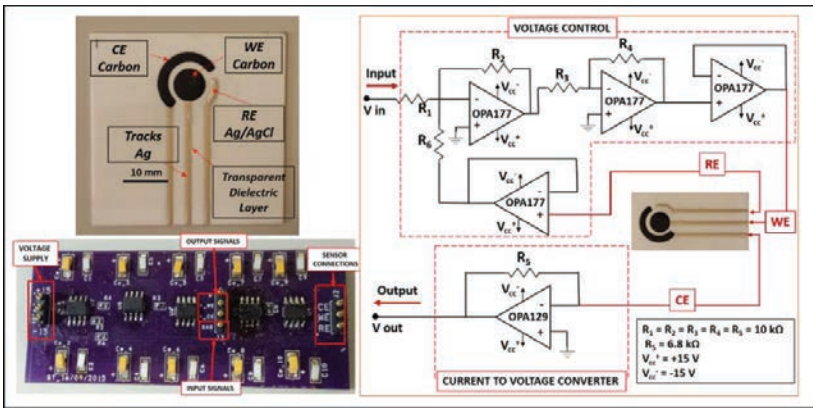

Figure 1: Final layout of the Biosensor and of the conditioning circuit

Parallelly, the conditioning and acquisition electronic circuit was designed in order to allow the production of a complete PoC testing platform. In particular, the design of the circuit was performed by following the scheme of common potentiostats. In these, a varying potential is applied to the WE with respect to the RE 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. Operational amplifiers were carefully selected depending on the specific function they had to perform in the circuit to ensure precision and accuracy.

2.2. Circuit testing using conductive solutions

The first test 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 between WE and CE. In the second test, 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 after an overnight coating. In both protocols, 2 ml of 5 mM $K_3[Fe(CN)_6]$ in 1 M KCl. were released on WE, CE and RE in order to allow current flow. Triangular waves were given as input at specific frequencies using a pulse generator and the output current flowing between WE and CE was recorded using an oscilloscope.

2.3. Anodic Stripping quantification of interleukin proteins

ASV protocol adopted for interleukin quantification in the calibration phase of the circuit was characterized by a specific functionalization of the WE using immucomplexes formed by a capture and a detection antibody, using a dedicated kit (DuoSet® development system for ELISA, Human CXCL8/IL-8). In order to perform the detection step, Alkaline Phosphatase (AP) was used to label the secondary antibodies, thus allowing a selective silver deposition, proportional to the amount of proteins detected. Finally, after placing a drop of buffer solution on the three electrodes, a ramp input was given to the WE, causing the oxidation of the deposited silver, and the current flowing between WE and CE was measured, allowing a sensitive quantification of the proteins.

3. Results

3.1. Circuit conditioning using conductive solutions

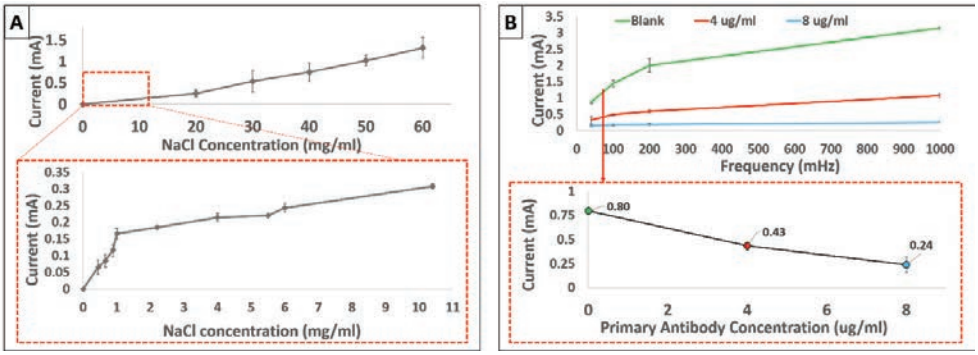

Figure 2: Circuit validation using saline solution (A) and quantifying antibodies coating concentration using ferricyanide solution (B)

Results from the evaluation of circuit response to changes in saline solution conductivity showed a particular trend, characterized by two different slopes respectively for concentration of NaCl lower and higher than 1.0 mg/ml.

In particular (Fig.2A), a higher sensitivity was shown for concentrations lower than 1.0 mg/ml, (0.2 mA/(mg/ml)) and a lower sensitivity (20 µA/(mg/ml)) for higher concentration, probably due to the specific features of the SMD

components chosen, which makes them really precise and sensitive to detect small changes in ionic current. 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. 2B). The sensitivity of the sensor in detecting the change in antibodies coating concentration was of 80 µA/(µg/ml).

3.2. Anodic Stripping quantification of interleukin proteins

Results from ASV measurements showed an increase in the maximum peak of current proportional to the increase of interleukin concentration. A nearly linear increase of current peaks could be observed, with a sensitivity of 45 µA/(ng/ml) (Fig.3).

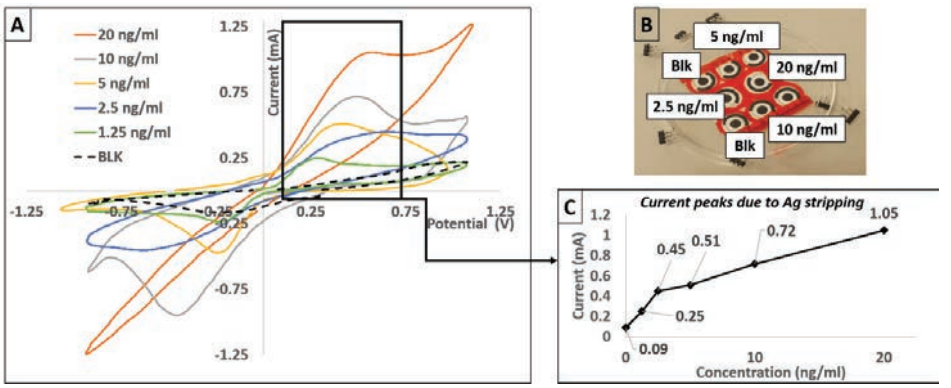

Figure 3: Circuit calibration quantifying interleukin using Anodic Stripping Voltammetry: Cyclic Voltammogram (A); Multiwells SPES (B); Current peak due to silver oxidation (C).

4. Conclusion

The results obtained showed good reproducibility, reliability and sensitivity, promising for the future developments with lower values of proteins concentrations. On-going activity refers to further investigation on sensor variability, essential in order to proceed with the quantification of unknown concentration of unfolded p53 protein. 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. All this, with the aim to realize an innovative low cost, sensitive and portable point-of-care, able to support clinicians to diagnose AD from its earliest stages.

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