ALL INKJET PRINTING SENSOR DEVICE ON PAPER: FOR IMMUNOSENSORS APPLICATIONS.

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ABSTRACT

A novel flexible, low-cost and miniaturized paper-based transducer has been developed. It consisted of an array of three microelectrodes where, the working and counter microelectrodes were easily printed using a gold nanoparticle ink, whereas a silver nanoparticle ink is used to print a pseudo-reference microelectrode. Both inks are commercially available and can be sintered at low temperatures, starting already at 140 ºC, which allows the use of paper substrates. Using the transducer developed an immunosensor against cortisol was fabricated. Microelectrode characterization showed an enhanced of the electrochemical active area due to high roughness of paper. Which infers a high sensitivity of microelectrodes. Moreover, the micro-sensor developed demonstrated to be highly sensitive toward cortisol in the concentration range of 5 to 20 ng/ml.

KEYWORDS

Ink-jet, silane, paper-based, electrochemical, gold ink, silver ink, cortisol.

INTRODUCTION

Paper based analytical devices (PADs) are now in the spotlight for develop flexible, disposable, simpler and accurate devices. PADs typically comprise an arrangement of hydrophilic/hydrophobic microstructures patterned on paper substrates using techniques such as wax printing, photolithography or chemical vapor-phase deposition among others[1]. Electrochemical paper-based analytical devices (ePADs) provide more reliable measurements than single microelectrode detection or colorimetric sensors [2]. Therefore, they are increasingly used for applications in the biomedical, environmental, clinical analysis, food processing, and chemical industry fields [2], [3], [4].

Inkjet printing is a digital and non-contact printing technique approach that allows the mask-less deposition of different functional materials on rigid and flexible substrates. One of its wide range of application is to manufacture electrochemical sensors. Inkjet printing is currently at the foot of its own S-curve [6], more or less at the point where screen printing was 20 years ago. In one hand, this means that new applications can be expected to appear exponentially within the next few years as the technology consolidates in manufacturing [7]. On the other hand. electrochemical disposable easy-to-use immunosensor are gaining a lot of attention in recent years.

Electrochemical immunosensing is based on the principle of measuring the changes in electrical properties of a conductive material due to the absorption of an analyte on the surface functionalized with antibodies [8]. Nowadays one of biomarkers gaining a lot of attention is Cortisol. Many efforts are putting to monitor the cortisol level in human body, due its relationship with many diseases between stress, Cushing syndrome, Adison's disease and even cardiac failure [9]. Electrochemical measurement gives us a reliable technique to quantify cortisol level in several kinds of samples [10]. Blood, plasma, serum, sweat and hair samples are well stablished for diagnosis several diseases, as it is well known these samples also give cortisol level.

The need of faster, low-cost, disposable, simpler and accurate devices has opened a new research field that focuses on simpler fabrication steps and quantitate results. To achieve this, we develop and all-inkjet printer device on a paper substrate.

MATERIAL AND METHODS

Materials and chemicals

For the development of the three-array microelectrodes, we used three commercially available inks. A low-curing gold colloidal ink (DryCure Au-J 1010B from Colloidal Ink Co., Ltd, Japan) was used for the working electrode (WE) and counter electrode (CE) development. A silver nanoparticle ink (Dupont-PE410 from USA) was employed for development of the pseudo-references (pRE) electrode, pads and tracks. The passivation and primer layer was done using SU8 ink (2002 from MicroChem, USA). The inks are formulated for the use of drop-on-demand inkjet technology. Whatman cellulose chromatography paper grade 1 (WHA3001861 from Merck) with a thickness of 0.18 mm with a linear flow rate (water) of 130 mm/30 min as substrate.

Phosphate buffered saline (PBS), potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$) and Potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6]$) (all from Merck, Spain) were used for activation and characterization of the printed microelectrodes. Hydrochloric acid (0.1 M) was electrochemically applied for the chlorination of the printed silver layers, and potassium chloride (KCl) for testing the open circuit potential of the pRE (both from Merck, Spain). 1H,1H,2H,2H-Perfluorooctyltriethoxysilane (from Merck, Spain) for the silanization of the substrate. 4-carboxymethylaniline (CMA), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride (EDC-HCl) and sodium chloride (NaCl) purchased from Acros Organics (France). Sodium nitrite

($NaNO_2$) was purchased from Merck. Hydrogen peroxide (H_2O_2), Sulfuric Acid (H_2SO_4) and Ethanolamine were from Fluka (France). Anti-Cortisol and hydrocortisone (cortisol) were purchased from Abcam (France).

Instrumentation

A piezoelectric Dimatix Material Printer (DMP 2831 from FUJIFILM-Dimatix, Inc., USA) was the printer used to print all the three inks on the paper substrate. The printer was used with fillable cartridge of 16 nozzles each with a diameter of 21,5 µm to get a 10 pL nominal drop volume. Printing patterns were made using Clewin 5 software and imported with the Dimatix bitmap editor software. The printing process was done in standard laboratory environment without temperature, humidity, particles control. Scanning electron microscopy (SEM, Auriga-40 from Carl Zeiss) was used to study the morphology of each printed layer and substrate treatments. The sheet resistance of conductive layer was measured with a Hewlett-Packard HP4155 Semiconductor Parameter Analyzer connected to a manual probe station PM5 (Suss Microtec GmbH). The contact angle was measured was done with Mobile Drop GH11 (Kruss GmbH) to confirm the hydrophobicity of the substrate.

The electrochemical characterization of the microelectrodes was performed with an 8-channel potentiostat 1030A Electrochemical Analyzer (CH Instruments, USA). Control experiments were performed using a commercial Ag/AgCl (3 M KCl) electrode as RE and a platinum electrode as CE both from (Metrohm, Germany). PalmSens4 (Palm Sens, Netherlands) was used to perform electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and chronoamperometric (CA) analyses after each step of the functionalization process.

Substrates treatment

In this work three different approaches were assessed to print the three-microelectrodes array on paper-based substrate: bare-paper, vapor silanization and primer.

Bare-paper was used as received with the only need of a high-pressure cleaning with a source of nitrogen. From now, this method will be known as “no treatment” method.

Silanization of paper makes it hydrophobic in order to get uniform lines of ink on the surface; the reaction occurs readily with the silanizing agent in the vapor phase and requires no equipment apart from a low-pressure chamber and a source of heat. The complete process is described in [11].

Conductive inks cannot be printed directly onto the porous substrate as this would cause short circuits and a non-defined electrode area. To electrically isolate electrodes from the lateral medium and get a smooth and non-porous surface, the porosity of the paper was sealed using a primer layer of SU8. The primer layer was locally printed just under the microelectrodes area blocking the minimal area, in order to not affect the properties of the substrates. With this strategy a conductive material can be obtained after the printing of the primer layer as is described in [12].

Ink-jet printing process

As afore mentioned, microelectrodes were printed on paper substrate. The paper sheet was placed on the stage of the DMP 2831 printer and heated up to a temperature of 40 ºC. Three individual ink cartridges were filled with about 1 ml of the different ink formulations (gold, silver and SU8). The drop ejection behavior of the inkjet nozzle was visually checked using the integrated drop-watcher camera to ensure reliable process conditions. Printing and curing process are described in [13]. After all the electrode were printed, we obtain a WE of a 0.78 μm^2 area. Finally the printed silver microelectrode was chlorinated by CV in 0.1 M HCl, scanning the potential from 0 V to 0.2 V versus Ag/AgCl commercial RE at 20 mV/s to obtain a stable Ag/AgCl pRE [14]. The procedure for the fabrication of the three-microelectrodes array is illustrated in Fig. 1.

First, we printed directly onto the bare paper after a fast cleaning process with high pressure nitrogen gun to blow all big particles on the surface. Then we develop the the three-electrode array after printing a primer layer of SU8 [15]. Finally silane was evaporated in order to get hydrophobic surface where the inks can be printed easily [11]. Detailed printing process is highly described in [12][13].

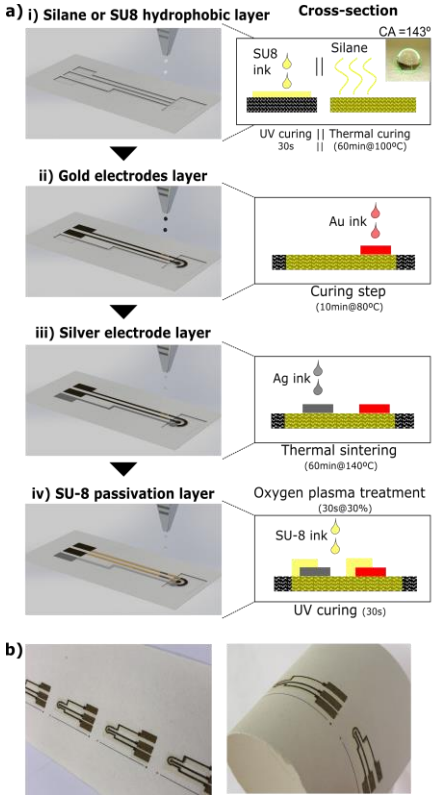

Figure 1: a) Fabrication steps of the three-electrode array based on inkjet printing with printing of the primer layer or evaporation of silane, printing of the gold and silver elements and printing of the passivation layer after thermal sintering and plasma treatment, b) photographs of the final device.

Functionalization of WE surface

The functionalization of bare gold WE was carried out on several steps. Initially, the gold microelectrode was activated by amperometric pulses (5 pulses of 5 s from 0 to -0.2V) on PBS. CMA solution was prepared in water at a concentration of 3mM. Afterwards, a mixture of 15 mM of HCl and 15 mM $NaNO_2$ was added to create the diazoted derivative that was electrodeposited onto gold

microelectrode by CV. For this purpose, six cycles were applied to the gold WEs at a potential range of -1.2 to 0.2 V and scan rate of 50 mV/s. Subsequently, the carboxylic acid groups of CMA were activated with EDC-NHS and then immediately incubated in PBS solution containing Anti-Cortisol at 2.5 µg mL^{-1} for 2 h at 4ºC. The functionalization was previously described in [16].

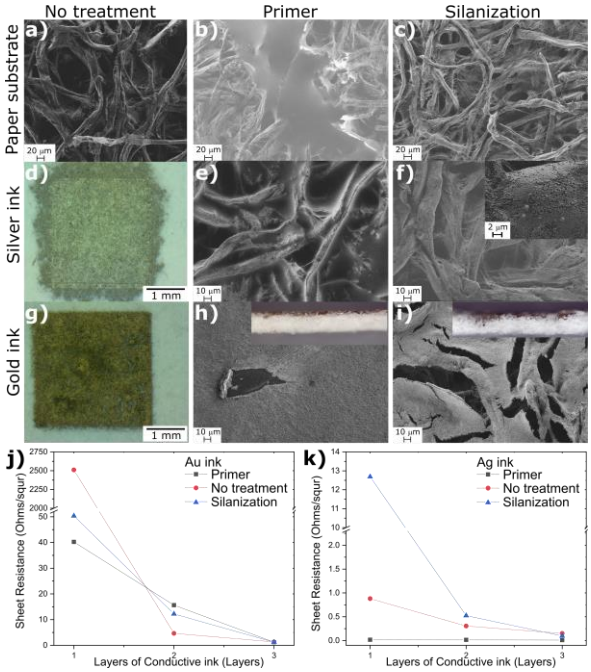

Figure 2: SEM images of paper no treatment a) primer b) silanized c), silver ink on paper no treatment d) primer e) silanized f), gold ink on paper no treatment g) primer h) silanized i). j) and k) sheet resistance vs numbers of layers deposited.

RESULTS & DISCUSSION

Morphological and electrical characterization

The morphology and topography of the printed layers were also studied. Fig. 2 shows the SEM images of the inkjet-printed gold, silver and SU8 ink after the sintering process onto the paper substrate. These images demostrate that after the curing process, the particles are agglomerated and a continuous conductive network is formed. For the silver ink, it looks that nanoparticle coated the fibers of the paper substrate but still form continous network. On the other side, the gold ink formed a monolayer onto the surface with enormeous cracks that affect negativily the conductivity of the material.

Fig. 2j and 2k shows the measured sheet resistance versus the number of layers of conductive ink for the gold and silver inks onto the three different treated substrates. Both inks were sintered at 140 ºC for 60 minutes. For the gold ink we got high resistance in 1 and 2 layer for silanization and primer treatments while the other cases showed low resistance that allowed us to work with it. Meanwhile the silver ink presented high resistance just for 1 layer onto silanization treatment. In order to get best and reproducible results, we decided to use in the three subtsrate treatments the method based on 3 layer of gold ink and 2 layers of silver ink.

Electrochemical response

CV is a commonly used technique for comparing and studying the behavior of the microelectrodes. The electrochemical reversible ferri(III)/ferro(II)-cyanide redox couple $[Fe(CN)_6]^{3-} + e^- \leftrightarrow [Fe(CN)_6]^{4-}$ was chosen as a model analyte for the CV experiments carried out. Fig. 3 (inset) shows the CV obtained by measuring the current at the WE before and after its activation. As can be seen, the optimized electrochemical activation is very important to ensure a good performance of the microelectrodes in three cases. Fig 3 shows the CV of three microelectrodes printed onto the three different treated substrates using in this case external CE and pRE. The anodic/cathodic peak current values (Ip) are directly proportional to the WE electrochemical active area as shown in the theoretically Randles-Sevcik equation. To determine if the microelectrodes are working properly, we compared the obtained Ip with the theory, using Randles-Sevcik's equation [17] showed in Fig. 3. Results in Fig. 3b shows different values of Ip for each microelectrode. It can be explained due their 3D structure onto the substrate, as can we see in Fig. 2h gold ink on primer shows the most planar structure among them having the lower Ip current, while gold ink printed on silanized paper showed higher Ip due to the enlarged surface area characteristic of 3D structured nanomaterial. These results show another advantage of the use of inkjet printing technology giving to microelectrodes the rugosity to almost tripling the electrochemical active area of a microfabricated planar bare gold microelectrode.

CVs were repeated using the printed CE and pRE. There were no differences between using the commercial CE or the printed CE. The small shift of the potential observed with the use of the integrated pRE-is attributed to the difference of chloride concentration in contact with the surface of the microelectrode.

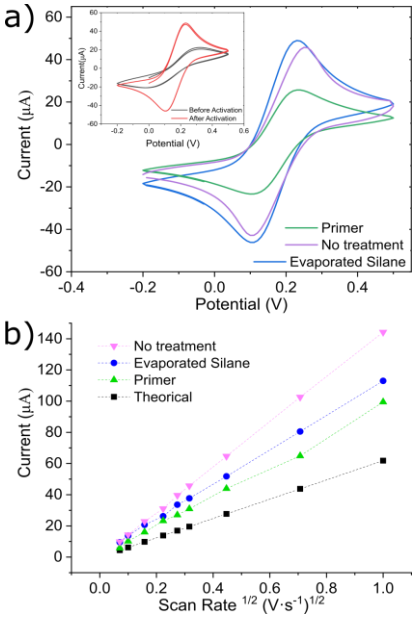

Figure 3: a) Cyclic voltammetry of three microelectrodes printed in the three different treated substrates. Inset shows the behavior of CV before and after activation. b) Correlation of the theoretical versus experimental Ip in function of square root of the scan rate using the Randle-Sevcik equation.

Cortisol detection

Preliminary results of detection of cortisol are show in Fig. 4. EIS was done in ferrocyanide redox couple to confirm the detection of cortisol with the developed ePAD.

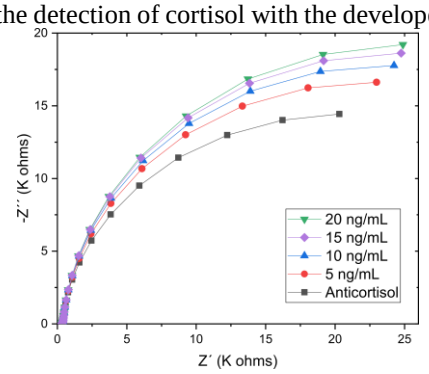

Figure 4: Nyquist impedance plot for cortisol detection.

CONCLUSION

The development and evaluation of a flexible, low-cost, disposable and miniaturized three-array microelectrodes transducer manufactured on paper substrate has been reported. Three different printing methods that used inkjet printing technology were assessed. The three of them resulted on a higher peak current than theoretical expected due high rugosity inks and surface. Experimental showed an increase of 2 folds in the electrochemical active area of the microelectrodes in the non-treated ones been the method with higher Ip. This fact proved the numerous advantages of the inkjet printing on paper substrate. Moreover, the microelectrodes were capable to be functionalized with anti-bodies against cortisol and preliminary results showed that the immunosensor worked perfectly in the concentration range of 5 to 20 ng/mL of Cortisol. This immunosensor represents a promising low-cost, disposable and reliable device for the accurate quantification of Cortisol in salivary and sweet samples.

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