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Glucose sensing electrode system based on polymeric microneedles

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***Abstract*— Microneedles are largely applied in biomedicine, both in diagnostics and therapeutics, since they can be considered the perfect interface between patient and sensing/dispensing devices. In this paper, we present an electro-chemical biosensor based on polymeric microneedles array for the measure of glucose level in interstitial fluids. The microneedles array has been fabricated by photolithography of a polymer, PolyEthylene Glycol DiAcrylate hydrogel, mixed with an enzyme, Glucose oxidase, and a redox mediator, the Vynilferrocene. The microneedles array acts as a working electrode when gold plated by sputtering and their tips have been etched to create a sensitive area. The redox reaction with glucose creates a charge transfer, enhanced by the redox mediator, resulting in a current proportional to the glucose concentration.**

Keywords—Microneedles; glucose sensor; microfabrication;

I. Introduction

In the age of smart phones and smart watches, the integration of health monitoring devices is largely used to commercially promote new models. However, low invasiveness constraint of such devices prevents the monitoring of substances such as glucose, although diabetes is one of the most widespread ailments. Microneedles (MNs) are widely applied as theranostic devices, since they are the perfect painless interface between the patient and a sensor or a dispenser [1-4]. In this paper, we present a multi-analyte platform based on tree electrodes system integrating polymeric MNs arrays for the measure of analyte level in the subcutaneous interstitial fluid. MNs arrays have been fabricated by photolithography of a PolyEthylene Glycol DiAcrylate (PEGDA) hydrogel mixed with the enzyme, Glucose oxidase (GOx), and a redox mediator, Vynilferrocene. The PEGDA hydrogel avoids fast denaturation of enzyme and allows analyte molecules propagation inside the MNs [5]. As reported in ref. [5], enzymes encapsulated in PEGDA hydrogel showed linear response up to 20 mM in case of glucose. However, the system presented in ref. [5] was not ready for direct measurements on patients. On the other hand, hydrogel liquid nature allows casting and direct polymerization, and, as a consequence, eliminates the etching step in the fabrication process and drastically decreases cost of production [6]. In particular, in our recent work [6], we have shown the possibility to tune shape and length of MNs by simply modifying the photolithographic parameters. In the present work, PEGDA is employed to trap the enzyme in a 3D MNs array. Moreover, when sputtered by a thin layer of gold each MN acts as a working electrode. In principle, each MN shaped working electrode could include a proper enzyme for a selective sensing of different analytes. As a proof of concept, we present the fabrication and the experimental characterization of a single working electrode containing GOx and Vynilferrocene (VF) molecules, as redox mediator. The redox reaction of GOx with glucose creates a charge transfer, mediated by the VF, resulting in a current proportional to glucose concentration.

II. Materials and Methods.

A. Materials

All chemicals are commercially available and used as received. PEGDA (average molecular weight Mn=250), phosphate buffered saline (PBS), gold etchant standard (etch rate 2.8 nm/sec @ 25 °C), Vynilferrocene (VF), Magnesium chloride ($MgCl_2$), D-(+)-glucose and Glucose oxidase (GOx) have been obtained from Sigma Aldrich. The photoinitiator 2-hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur© 1173) has been purchased from BASF. Gold target used for the sputter deposition has been furnished by Emitech. D-(+)-glucose solutions in PBS have been stored overnight at room temperature before use, in order to allow muta-rotation equilibrium.

B. Redox reaction

The redox reaction between D-glucose and Gox is well known in literature and transduces the analyte concentration into an electrical current value. The GOx enzyme, with the redox center flavin adenina dinucleotide oxidized (FAD), is an oxido-reductase that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-1,5-lactone [7]. The reaction in presence of VF is the following:

$$\beta\text{-D-glucose} + \text{GOx(FAD)} \rightarrow \text{D-glucono-1,5-lactone} + \text{GOx}(FADH_2)$$

$$\text{GOx}(FADH_2) + 2\text{vinylferrocene}_{ox} \rightarrow \text{GOx(FAD)} + 2\text{vinylferrocene}_{red} + 2H^+$$

$$2\text{vinylferrocene}_{red} \rightarrow 2\text{vinylferrocene}_{ox} + 2e^-$$

where FAD is flavin adenine dinucleotide oxidized and $FADH_2$ is flavin adenine dinucleotide reduced. The presence of a redox mediator enhances the electronic transfer from the reaction site (enzyme-analyte) towards electrodes.

C. MNs array Fabrication

The fabrication process of the MNs array is shown in the sketch in Fig. 1 and follows what reported in ref. [6], except for the PEGDA based mixture used. Briefly, a basic solution of PEGDA and DAROCUR© at 2% volume/volume concentration has been used to fabricate the substrate support and the MNs.

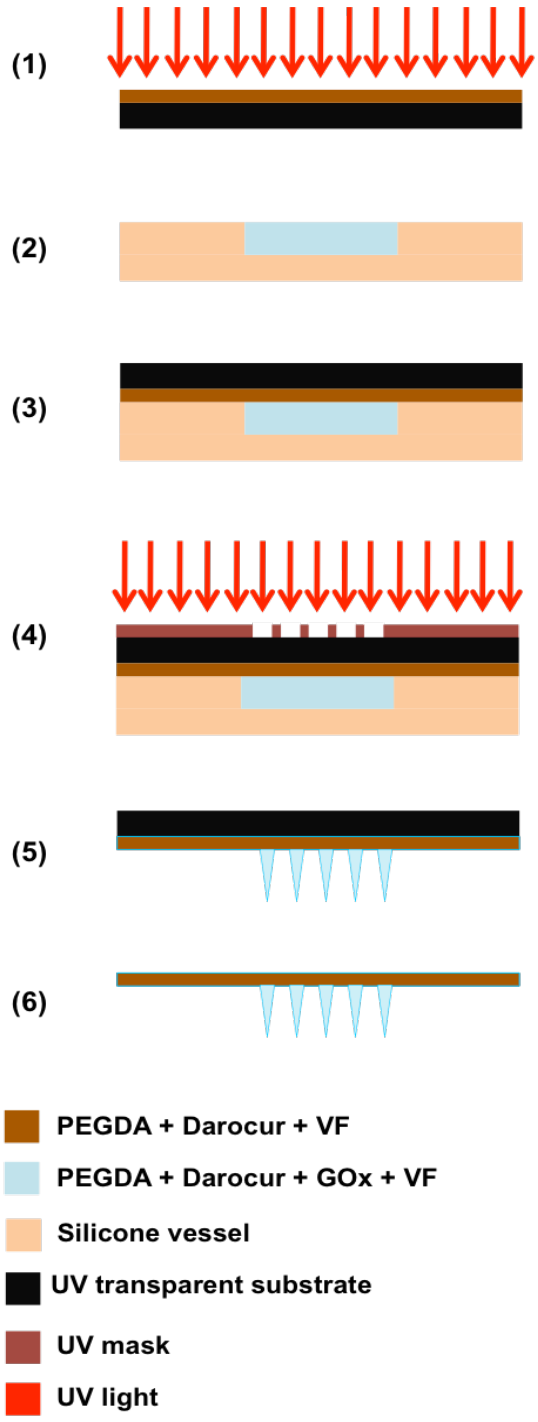

Fig. 1. Flow chart of the fabrication process of the microneedles array (sketch not in scale). (1) Creation of a layer of PEGDA + Darocur + VF on glass slide. (2) Filling of the silicone vessel with the solution constituted of PEGDA, Darocur, GOx and VF. (3) Overturning substrate to close the silicone vessel. (4) Exposure to UV light. (5) Development in water. (6) Removing of the glass slide.

For the substrate layer, the VF (1% in weight/volume) has been added to the basic solution. This solution has been casted on a UV transparent support and exposed at UV light without any photomask (see the first step in Fig. 1). The DAROCUR© initiates the polymerization of the PEGDA hydrogel, by exposing the solution at UV light. A molecular sketch of the polymerization scheme is shown in Fig. 2. In this scheme, the VF molecules are trapped into the polymeric matrix without bonding it.

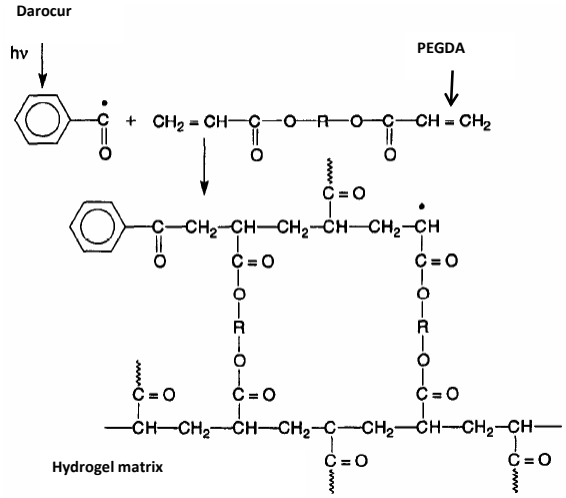

Fig. 2. Molecular sketch of the polymeric matrix obtained by the PEGDA and Darocur reaction.

For MNs, a solution of VF (1% in weight/volume) and GOx (in PBS pH 6.0 at 20 mg/mL concentration) has been mixed with the basic solution in ratio 1:10. The quantity of 1.2mL of this solution has been casted into a silicone vessel which volume is $(4x20x15)mm^3$ (see the second step in Fig. 1). After overturning the substrate layer with the transparent support on the vessel (step 3 in Fig. 1), the solution has been exposed to UV light trough a photomask with a holes array. The UV light propagates through the holes in the mask, the UV transparent layer and the support layer and it partially exposes the solution containing the enzymes (step 4 in Fig. 1). The MNs shape follows the UV light cone with higher intensity. Finally, the MNs are developed in deionized (d. i.) water (step 5 in Fig. 1) and removed by the UV transparent support (step 6 in Fig. 1).

D. Electrode Fabrication

Fig. 3 shows the flow chart of fabrication process of the working electrode (sketch not in scale). A thin film of gold has been deposited on the MNs array in order to create the electrical contact (see the first step in Fig. 3). Gold has been deposited at room temperature by K975X sputter (by Emitech) with deposition time and current of 240 s and 30 mA, respectively, obtaining a 160 nm thick layer. In step 2 of Fig. 3, a thin layer of commercial gold etchant have been deposited on

a glass slide by spinning. In order to etch the tips, MNs have been overturned and put in contact with the gold etchant for 40s. Then, the MNs have been rinsed in d.i. water to stop the etching (step 3 in Fig. 3).

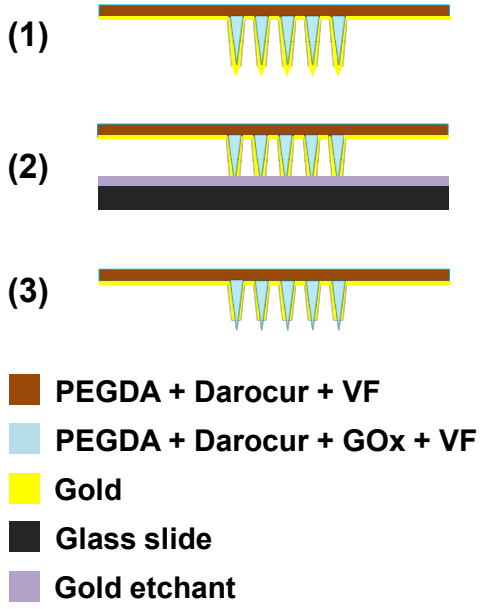

Fig. 3. Flow chart of the fabrication process of the working electrode (sketch not in scale). (1) Gold deposition on microneedles array. (2) Etching of the tips of the microneedles.(3) Rinsing in d.i. water.

III. Results and Discussion

The proposed system follows the structure of commercial devices for continuous and automatic glucose monitoring (CGM) and it has sketched in Fig. 4. This system combines very large scale low cost Silicon technologies, versatility and possibility of miniaturization in small devices with fast response and discrete view resulting in a significant improvement in the lives of millions of diabetics in the world, in particular elders and children. The system is based on the redox reaction between the analyte and the enzyme, which allows the direct conversion of the analyte concentration in an electrical current. The presence of a redox mediator speeds up the electronic transfer from the reaction site (enzyme-analyte) towards the electrodes. The key element is the material containing the enzyme: a biocompatible polymeric hydrogel-based PEGDA. The polymeric matrix of the solidified MNs embeds enzymes that can be locked or released when the PEGDA is soaked in aqueous solution, depending on the relative size of the enzymes and the polymeric cross-link. On the other hand, small molecules can enter into the swelled MNs and interact with trapped molecules. The uncovered tips allow the diffusion of the glucose molecules into the polymeric matrix.

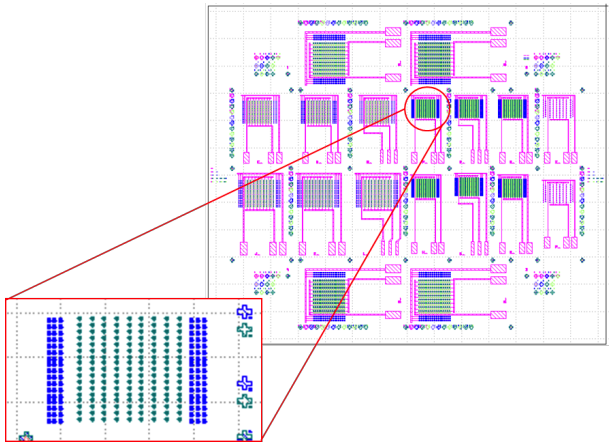

Fig. 4. Multi-analyte platform design containing 18 integrated electrochemical cells. Each electrochemical cell contains three electrodes: a platinum wire as auxiliary electrode, a saturated silver chloride (Ag/AgCl 3 M) as reference electrode and the microneedles based electrode as working electrode.

As a proof of concept, we present the fabrication results and the experimental characterization of a single working electrode containing GOx and having MNs array shape.

The electrode is shown in Fig. 5, where the flexibility of the substrate, the MNs array (A) and a single sensing MN (B) are reported.

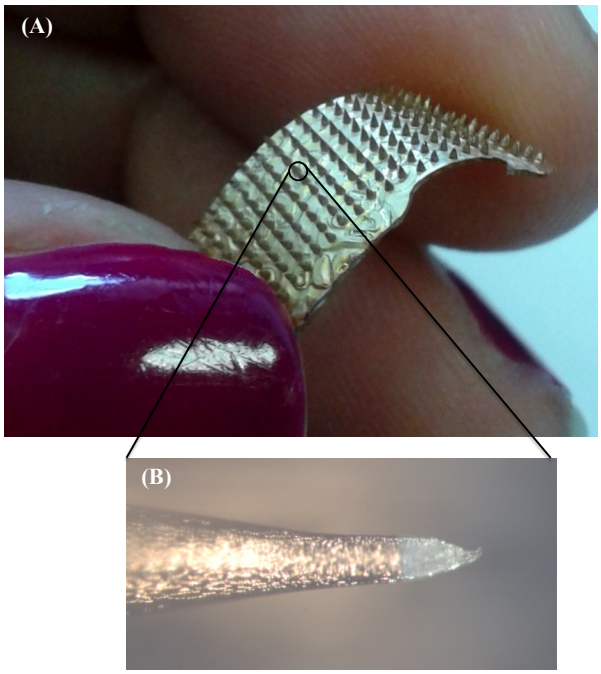

Fig. 5. (A) Picture of the working electrode. (B) Image of a microneedle with etched tip.

Inside the electrode, the entrapped enzyme is the glucose oxidase enzyme (GOx) (EC 1.1.3.4), an oxido-reductase that catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. During the oxido-reductase reaction, the production of electron can be monitored as current by applying a small potential (300mV).

Two kind of electrochemical characterizations have been performed: ciclic voltammetry and chronoamperometry. In both cases, the microfabricated working electrode is soaked in a electrolitic solution (PBS at pH 7.4 and 10 mM concentration with 0.48 mM of $MgCl_2$) together with a Ag/AgCl reference electrode and a platinum wire as auxiliary electrode in a three electrode cell, as sketched in Fig. 6.

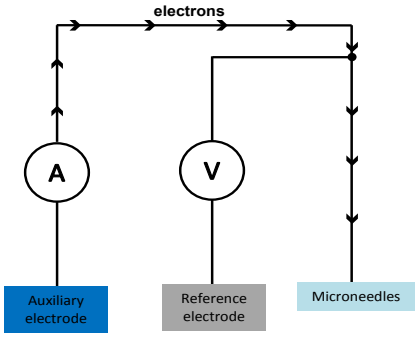

Fig. 6. Sketch of the three electrode cell: the working electrode is the microneedles based electrode, the reference electrode is the Ag/AgCl and the auxiliary electrode is the platinum wire. The working electrode is immersed in a electrolytic solution: the GOx enzyme, contained in the hydrogel matrix of the microneedles, interacts with the glucose contained in the electrolytic solution, with resulting in the generation of electrons as showed in the chemical reaction.

During the cyclic voltammetry characterization, the potential, applied between reference and working electrodes, has been scanned from -100 to 700 mV and backward, and the current in absence of analyte has been measured between working and auxiliary electrodes. This characterization verifies the effective functionality of the working electrode system that includes the non-conductive polymeric matrix. Cyclic voltammetry results are reported in Fig.7, where oxidation and reduction peaks related to redox of VF are well recognizable at 250mV and 200mV, respectively. This result indicates that the polymeric matrix slightly interferes on the kinetic of VF redox reaction. Moreover, the peaks have almost the same amplitude, than the redox reaction is almost reversible.

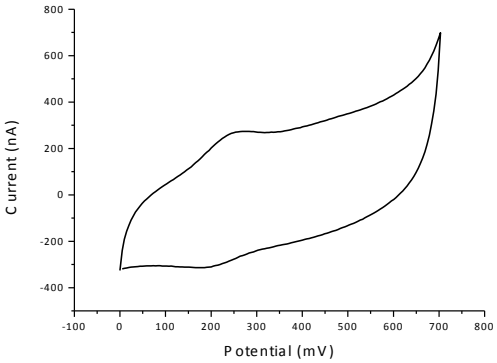

Fig. 7. Cyclic voltammetry curve characterizing the redox activity of vinylferrocene in presence of GOx in the hydrogel microneedles at scan rate of 50 mV/s.

Chronoamperometry is a characterization technique that reveals the relation between analyte concentration and electron production. During the chronoamperometric characterizations, the potential has been fixed at 300 mV and the current has been measured as function of time, in presence of known glucose concentrations. The resulting current falls in presence of glucose, at concentrations 0, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 5, 7, 9 and 12 mM, are reported in Fig. 8 (A).

As reported in Fig. 8, after a rapid fall the currents reach a value related to the glucose concentration.

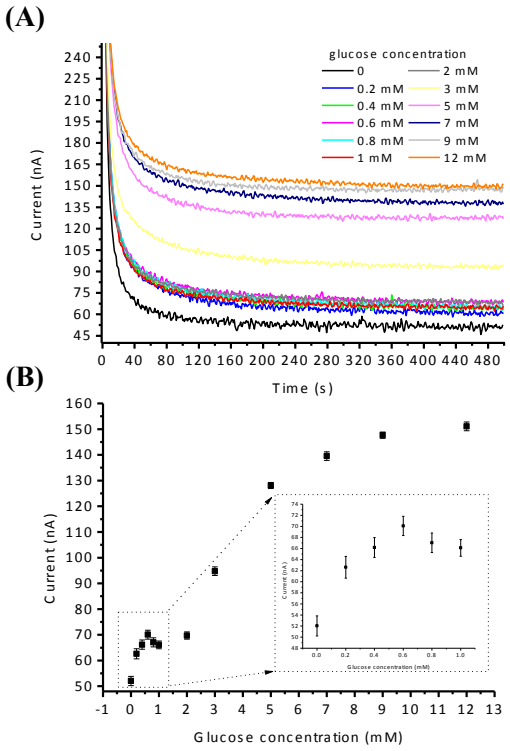

Fig. 8. (A) Chronoamperometric response and (B) calibration curve of the working electrode to several concentrations of glucose.

In Fig. 8 (B), the stabilization current in function of the glucose concentration has been reported. The linear range is for glucose concentration between 0 and 0.6mM, that is the range concentration suitable to monitor the glucose level in the interstitial human fluid. However, the stabilization time is about 120s that inhibits continuous monitoring, although is significantly slower than commercial POC. The present proof of concept opens to new possibilities of health monitoring. Future research is addressed to improve the speed of the measure by changing the molecular weight of the PEGDA, the VF and GOx concentration.

IV. Conclusions

A multi-analyte platform based on polymeric MNs can continuously and selectively monitor the analytes concentration with low invasiveness. It is conceived as array of working electrode, in turn conceived as array of MNs. As proof of concept, a single working electrode based on polymeric MNs and containing GOx has been fabricated and electrochemically characterized. The working electrode incorporating GOx molecules interacts with glucose reversibly, as proved by the cyclic voltammetry. Moreover, it is experimentally found that the polymeric matrix does not interfere on the redox reaction.

Finally the interaction results in a current proportional to its concentration, as reported by chronoamperometric characterization.

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