Sensors and Actuators B 236 (2016) 343–349

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Polymeric microneedles based enzymatic electrodes for electrochemical biosensing of glucose and lactic acid

A. Caliò[a,b,c], P. Dardano[a,b,*], V. Di Palma[b,d], M.F. Bevilacqua[b,d], A. Di Matteo[b,d], H. Iuele[b], L. De Stefano[a,b]

[a] National Research Council – Institute for Microelectronics and Microsystems, Via P. Castellino 111, 80131, Naples, Italy
[b] IMAST Scarl, Piazza Bovio 22, 80133, Naples, Italy
[c] University of Naples Federico II – Department of Physical Science, Via Cinthia, I-80126, Naples, Italy
[d] STMicroelectronics, Via Remo De Feo 1, 80022, Arzano, Italy

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 17 May 2016
Accepted 29 May 2016
Available online 1 June 2016

Keywords:
Microneedles
Photolithography
Polymer
Enzymes
Electrochemistry

ABSTRACT

Polymeric microneedles (MNs) based working electrodes are fabricated by standard photolithography of poly(ethylene glycol) diacrylate (PEGDA) doped by enzyme, redox mediator and photoinitiator. This flexible device acts as working electrode in electrochemical detection of glucose and lactic acid in solution when glucose oxidase (GOx) and lactose oxidase (LOx) enzymes, respectively, are used. Biosensor showed a linear response in the ranges from 0 to 4 mM and from 0 to 1 mM, for glucose and lactic acid, respectively. A limit of detection equal to 1 μM is found. The developed technology has been patented.

1. Introduction

A growing general attention on public health, in particular on nutrition and sport activity, indicated as primary prevention of diseases, such as obesity and diabetes as well as cancer, supports a continuous development of diagnostic devices in the last ten years. Even if blood pressure and heart rate monitors can be nowadays found integrated in cellular phones, the quantification of other relevant physiological parameters still requires dedicated instrumentation not available for self-diagnostic at home. In case of commonly tested analyte, such as the blood glucose level in patients affected by diabetes mellitus, several model of biosensors, based on GOx, are commercially available, also for unskilled person, but the measurement procedure is quite invasive and time consuming, in fact it must be repeated many times per day [1]. Another intermediate key substance in anaerobic glycolytic pathway metabolism is lactate, which is monitored in diseases such as obesity, diabetes, ischemia and cancer of digestive system [2–4]. The determination of lactate concentration in blood is also essential in sport medicine, where lactate levels are strictly monitored as marker of the training status [5]. Electrochemical enzyme-based electrodes exploit the redox reaction of an enzyme with an analyte, which creates a charge transfer, resulting in a current proportional to its concentration in blood: in case of lactate, the detection is due to the redox reaction of the LOx.

Enzymes are traditionally immobilized on electrodes and the binding procedure strongly influences the performances, the response time, the sensitivity, the stability and the lifetime of the sensor. Particular attention must be paid to avoid enzyme denaturation that could occur in case of nonspecific bond making the sensor insensitive. The enzyme immobilizing technique should promote a rapid propagation of generated electrons and avoid nonspecific binding and electrode failing. Electrode features can be optimized by using several matrices in which enzymes can be dispersed. Among many material science studies, hydrated gels appear excellent enzyme encapsulation materials, that avoid enzymatic binding, allow analyte molecules diffusion and interaction with the enzymes, provide near physiologic conditions circumventing enzymes denaturation [6]. In particular, devices made with electrodes modified by enzymes encapsulated in PEGDA hydrogel showed linear response up to 20 mM and 10 mM for glucose and lactate, respectively [6]. Moreover, the hydrogel nature of PEGDA permitted three-dimensional fabrication by casting and direct polymerization, avoiding etching step in fabrication process and drastically decreasing cost of realization [6–8]. In particular,

* Corresponding author at: National Research Council – Institute for Microelectronics and Microsystems, Via P. Castellino 111, 80131, Naples, Italy.
E-mail address: principia.dardano@na.imm.cnr.it (P. Dardano).

http://dx.doi.org/10.1016/j.snb.2016.05.156
0925-4005/

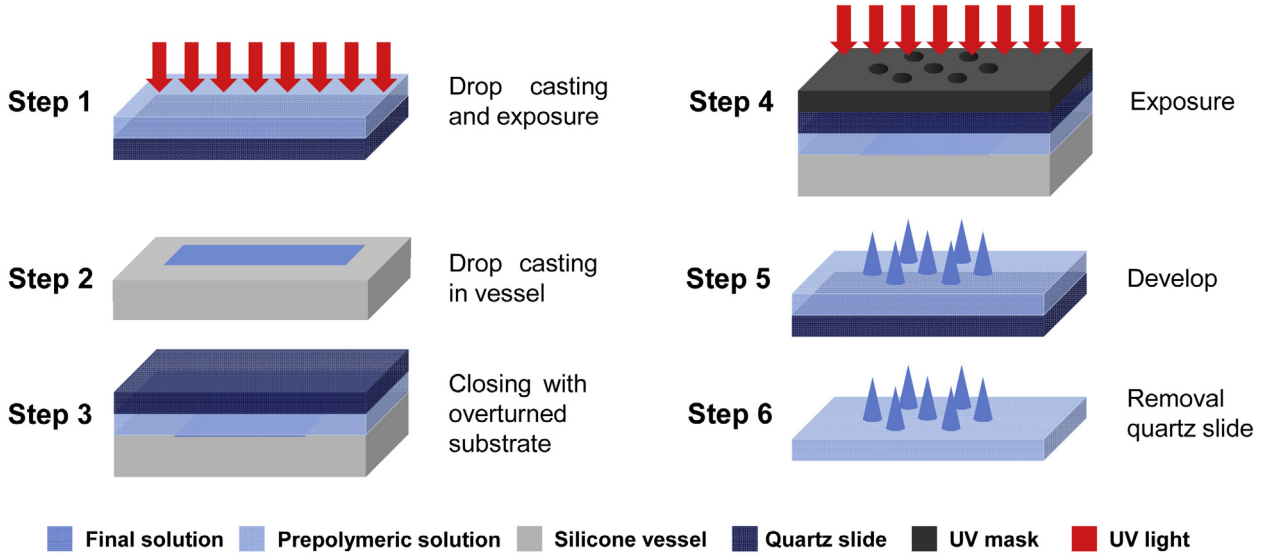

Fig. 1. Flow chart of the fabrication process of the MN array (sketch not in scale). Step 1: drop casting of prepolymeric solution on quartz slide and exposure to UV light. Step 2: filling of silicone vessel by casting the final solution. Step 3: overturning PEGDA layer to seal the silicone vessel. Step 4: exposure to UV light through photomask in soft contact mode. Step 5: development in deionized water and rinse with nitrogen. Step 6: removal of quartz slide by tweezers.

the three-dimensional micro-objects of PEGDA have been realized such as MNs of several length, shape and tips, which can be very useful for biomedical applications [7–9].

Contrarily to commercially available blood biosensors, which require at least millimetric needle or blade to open skin till leakage of a blood droplet, MNs based biosensor could be considered perfect painless interface between the patient and a sensing device [10–15]. Indeed, MNs overcome the primary physical barrier, i.e. the stratum corneum, without pain, also satisfying the minimum invasiveness demand, strictly required for children and elder patients [16]. Hydrogel MNs have been proposed in local drug delivery, since traditional transdermal delivery systems are limited to small and lipophilic molecules. MNs devices can release hydrophilic large molecules directly to interstitial liquid, which is in osmotic equilibrium with the blood concentration [17,18]. In the field of sensing application, the osmotic equilibrium of analyte partial pressure between blood and interstitial liquid is exploited in combination with the osmotic equilibrium between interstitial liquid and hydrogel to quantify analytes concentration [19].

In this paper, we report on a multi-analyte biosensor platform based on three electrodes system constituted by a polymeric microneedle (MN) array for the measure of glucose and lactic acid level in subcutaneous interstitial liquid. The working electrodes, in the form of MN array, are fabricated by photolithography of a mixture of PEGDA hydrogel, photoinitiator Darocur©, enzyme GOx or LOx, and redox mediator vinylferrocene (VF). The redox reaction of GOx (LOx) with glucose (lactic acid) results in a charge transfer, mediated by VF, producing a current proportional to analyte concentration. Small dimensions, low energy consumption, easy fabrication method and very low invasiveness make the presented biosensor really competitive compared to current devices. Moreover, biosensing structure could be easily integrated in traditional patch systems, as well as commercial wearable technology devices.

2. Experimental

2.1. Materials

All chemicals were reagent grade or higher and have been used as received unless otherwise specified. PEGDA with average molecular weight (M_n) of 250, phosphate buffered saline (PBS) 10 mM (NaCl 0.138 M, KCl^- 0.0027 M) at pH 7.4, VF, magnesium chloride ($MgCl_2$) 0.48 mM, D-(+)-glucose, GOx (from *Aspergillus niger*, type II, ≥15,000 units/g solid, EC 1.1.3.4), L-(+)-lactic acid and LOx (from *Pediococcus* sp. lyophilized powder, ≥20 units/mg solid, EC 1.13.12.4) have been obtained from Sigma Aldrich, USA. The photoinitiator, 2-hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur© 1173), has been acquired from BASF, Germany. Gold used for the sputter deposition came by Emitech, Italy. Silicone rubber has been taken from Saratoga, Italy. All aqueous solutions have been prepared with ultrapure water by means of a MilliporeMilli-Q system (18.2 MΩ cm). Stock solutions of glucose and lactic acid have been prepared in PBS solution and stored at 4 °C in the dark. In order to reach sugar muta-rotation equilibrium, D-(+)-glucose solutions have been stored overnight at room temperature before using.

2.2. Redox activity of glucose and lactate oxidase enzymes

Sensing mechanisms, described by Eqs. (1)–(3) for glucose and (4)–(6) for lactic acid, is based on redox reactions between analytes (glucose or lactic acid) and enzymes (GOx or LOx), mediated by an electron-carrying molecule (vinylferrocene) able to carrier produced electrons from the redox center of the enzyme to the surface of the electrode, as reported in details in these Refs. [1,15].

$$glucose + GOx_{oxid} \rightarrow gluconicacid + GOx_{reduc} \quad (1)$$

$$GOx_{reduc} + 2M_{oxid} \rightarrow GOx_{oxid} + 2M_{reduc} + 2H^+ \quad (2)$$

$$2M_{reduc} \rightarrow 2M_{oxid} + 2e^- \quad (3)$$

$$lacticacid + LOx_{oxid} \rightarrow pyruvicacid + LOx_{reduc} \quad (4)$$

$$LOx_{reduc} + 2M_{oxid} \rightarrow LOx_{oxid} + 2M_{reduc} + 2H^+ \quad (5)$$

$$2M_{reduc} \rightarrow 2M_{oxid} + 2e^- \quad (6)$$

GOx_{oxid}, LOx_{oxid}, M_{oxid} and GOx_{reduc}, LOx_{reduc}, M_{reduc} are GOx, LOx and redox mediator M in oxidation and reduction states. Through these reactions, the analyte concentration (proportional to electrons transferred) is transduced into an electrical current value.

2.3. Microneedles based enzymatic electrodes fabrication

MNs based enzymatic electrodes have been achieved by gold-coating MN array, containing the enzyme and the redox mediator. Two working electrodes have been realized, one for the detection of glucose and the other for lactic acid. They have been fabricated by photolithography and sputter deposition in clean room ISO 5.

2.3.1. Microneedle array fabrication

The fabrication process of MN array is showed in Fig. 1 and is based on the process deeply described in a recently published paper [7].

In order to realize two different MN arrays, one for the detection of glucose and the other for the lactic acid, two specific solutions, so called final solutions, were used as photoresists in the photolithographic process. They were obtained by mixing 90% v/v of the pre-polymeric solution, which is the same for both MN arrays, and 10% of proper enzymatic solution. The pre-polymeric solution was prepared by adding 2% v/v of Darocur© and 1% w/v of VF to liquid PEGDA. The two enzymatic solutions were made of GOx in PBS (pH 6.0) at 20 mg/mL concentration and LOx in PBS (pH 6.5) at 15 mg/mL concentration. The enzyme concentration used was the same of that given in Ref. [6]. By mixing pre-polymeric and proper enzymatic solutions, the two final solutions have been obtained. Both final solutions, were stirred for 4 h at 4 °C to get homogeneous dispersion of enzyme molecules, as reported in Ref. [6]. These two final solutions behaves as negative photoresists, i.e. on exposure to UV light they became crosslinked/polymerized and much less soluble in the developer solution than unexposed ones.

An amount of 1 mL of pre-polymeric solution was deposited on quartz slide by drop casting technique and exposed to UV light for 10 s (Fig. 1, step 1). The exposure steps have been performed with MA6/BA6 mask aligner (by Karl Suss AG, Germany) at 365 nm wavelength. The obtained PEGDA layer was about 1 mm thick and it was used as flexible support for the MN array. A silicone vessel ($0.4 \times 1.0 \times 1.5$ cm × cm × cm) was fulfilled with the final solution (Fig. 1, step 2), once with solution containing GOx and the other with that containing LOx. After this step, the PEGDA layer on the quartz slide was overturned and placed on the silicone vessel to close it (Fig. 1, step 3). The direct contact between the final solution and the PEGDA layer allowed the adhesion of the MN array on the PEGDA layer. The UV exposure was performed at 450 mJ/cm^2 (for 25 s at 18 mW/cm^2) through a quartz/chrome photomask in soft contact mode (Fig. 1, step 4). Samples of GOx or LOx based PEGDA matrix was developed in deionized water for 2 min, in order to remove the unpolymerized solution, and dried with nitrogen (Fig. 1, step 5). Finally, the MN array on the PEGDA layer was removed from the quartz support by means of tweezers (Fig. 1, step 6).

2.3.2. Electrodes fabrication

Fabrication steps of the two working electrodes are shown in Fig. 2.

In order to electrically contact the MN array, a gold layer was deposited on the back of the PEGDA layer. Metal deposition was performed by the K975X Turbo-Pumped Thermal Evaporators equipped with EK4175 Sputtering module (by Emitech, Italy) at room temperature with a current of 30 mA, at operating vacuum 1×10^{-5} mbar and a deposition time of 240 s (Fig. 2, step 1), obtaining a 160 nm thick layer of gold. The gold layer was covered by a thin layer of commercial liquid silicone rubber (Saratoga, Italy), hardened at room temperature in 2 h (Fig. 2, step 2). The silicon rubber film on the back of the gold layer prevented direct contact between the electrode and the electrolytic solution during the electrochemical characterizations. As shown in Fig. 2, a small area of the gold layer remained uncovered by silicone, in order to allow the electric contact with the electrochemical cell setup. However, the uncovered gold was not immersed into the electrolytic solution during electrochemical measurements.

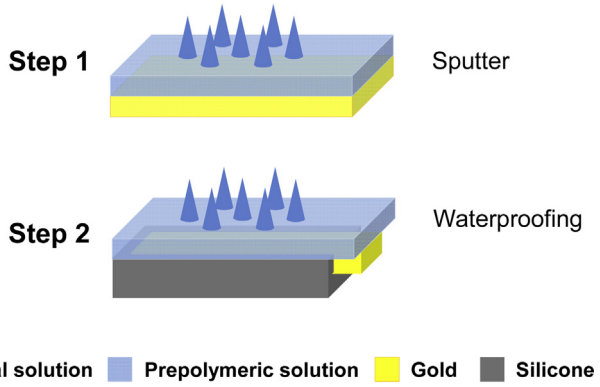

Fig. 2. Flow chart of the fabrication process of the working electrode (sketch not in scale). Step 1: gold sputtering on the back of MN array. Step 2: waterproofing of the gold layer by hardened liquid silicone rubber. Note that the gold layer was slightly uncovered by hardened silicone rubber in order to electrically contact the working electrode.

2.4. Electrochemical measurements

To provide a working proof of concept of the biosensor, in vitro test, as reported in Ref. [6] have been performed. To this aim, a three electrodes BASi C-3 Cell Stand has been used: a platinum wire auxiliary electrode, a saturated silver chloride (Ag/AgCl 3 M) reference electrode and the MNs based enzymatic electrode (that for glucose or that for lactic acid) as working electrode. Moreover, PBS (pH 7.4) mixed with $MgCl_2$ was used as electrolytic solution to mimic the interstitial liquid. The electrolytic solution was deoxygenated with nitrogen during the measurements. All electrochemical measurements were performed at room temperature. Cyclic voltammetry (CV) and chronoamperometry (CA) techniques have been used to characterize the working electrodes. In particular, the CV measurements revealed the activity of the redox mediator, the influence of the PEGDA polymeric matrix on its redox kinetic and on its reversibility. Moreover, the CA gave information on the influence of the analyte concentration on the electrons production.

During the CV measurements, the potential applied between the working electrode and the reference electrode was linearly changed and the current between the working electrode and the auxiliary electrode was measured. For working electrode containing GOx, the potential varied from −100 to 700 mV, and backward, with scan rates 30, 50, 70 and 100 mV/s. For working electrode containing LOx, the potential varied from −100 to 600 mV, and backward, with scan rates 30, 50, 70 and 100 mV/s.

During CA measurements, the potential between working and reference electrodes were fixed and the current, flowing from working to auxiliary electrodes, was measured as function of time. The CA characteristics were performed 5 min after addition of aliquots at known concentration of analyte (glucose or lactic acid), in order to allow the diffusion inside the electrolytic solution, at 300 mV. Glucose and lactic acid were detected at the following concentrations: 0, 2, 4, 6, 8, 10, 12 mM and 0, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 5, 7, 9, 12 mM, respectively.

3. Results and discussion

The proposed systems can be considered as proof-of-concept of locally skin-implanted electrochemical biosensors, useful for monitoring of glucose and lactic acid levels in the interstitial fluid. The photolithographic approach to MN array fabrication, based on photocurable hydrogels, has been recently reported in literature [7,20]:

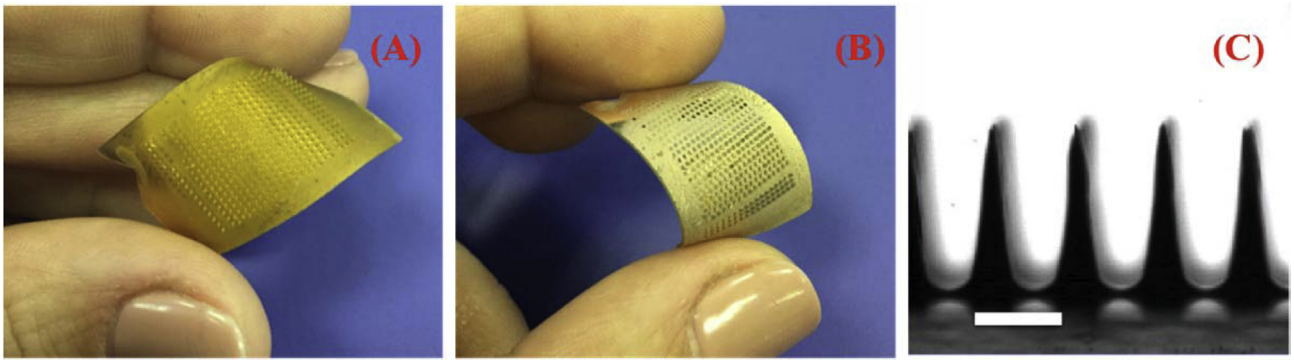

Fig. 3. Pictures front (A) and back (B) of the MNs based enzymatic electrode acquired by digital camera. (C) Image of the MNs arrays captured by the Water Contact Angle camera (scale bar is 400 μm).

the use of very large integration compatible technology in realization of large consume devices guarantees low cost, which is highly required for healthcare products. Moreover, by changing few process parameters, namely exposure time and optical power, MNs of different shapes and length can be simply obtained. Nevertheless, it was not trivial to apply and optimize the fabrication procedure when the polymer was mixed with biological and chemical agents. Fig. 3 shows a photo of the MN array working electrode: the device was completely flexible, in particular the MN array (Fig. 3(A)) could be deformed without breaking, and the back resulted continuously covered by gold (Fig. 3(B)), assuring good electrical contact.

The length of the MNs in the picture was of about 500 μm. Beside flexibility, a polymeric matrix had several functions and advantages with respect to other materials, mainly the harder ones (i.e. silicon and glass): its nanoporosity could be exploited for stabilizing the enzymes and filtering in size the analytes; in this way, the enzymes were confined inside the polymeric matrix, but the electrolytic solution containing the analyte molecules can enter the material and interact with the enzymes.

A scheme of the device working principle and experimental setup geometry are illustrated in Fig. 4: the MNs based electrode (Fig. 4(A)) was immersed in the electrolytic solution contained in the three electrode cell. Polymer swelling made analyte molecules (glucose or lactic acid) penetrate into the sensitive area (Fig. 4(B)) and interact with the enzyme (GOx or LOx) locked inside the porous matrix (Fig. 4(C)), resulting in the generation of electrons (see redox reaction) current collected by auxiliary electrode.

A first characterization of the two devices monitored the redox activity of the VF mediator in presence of the enzymes (GOx or LOx)

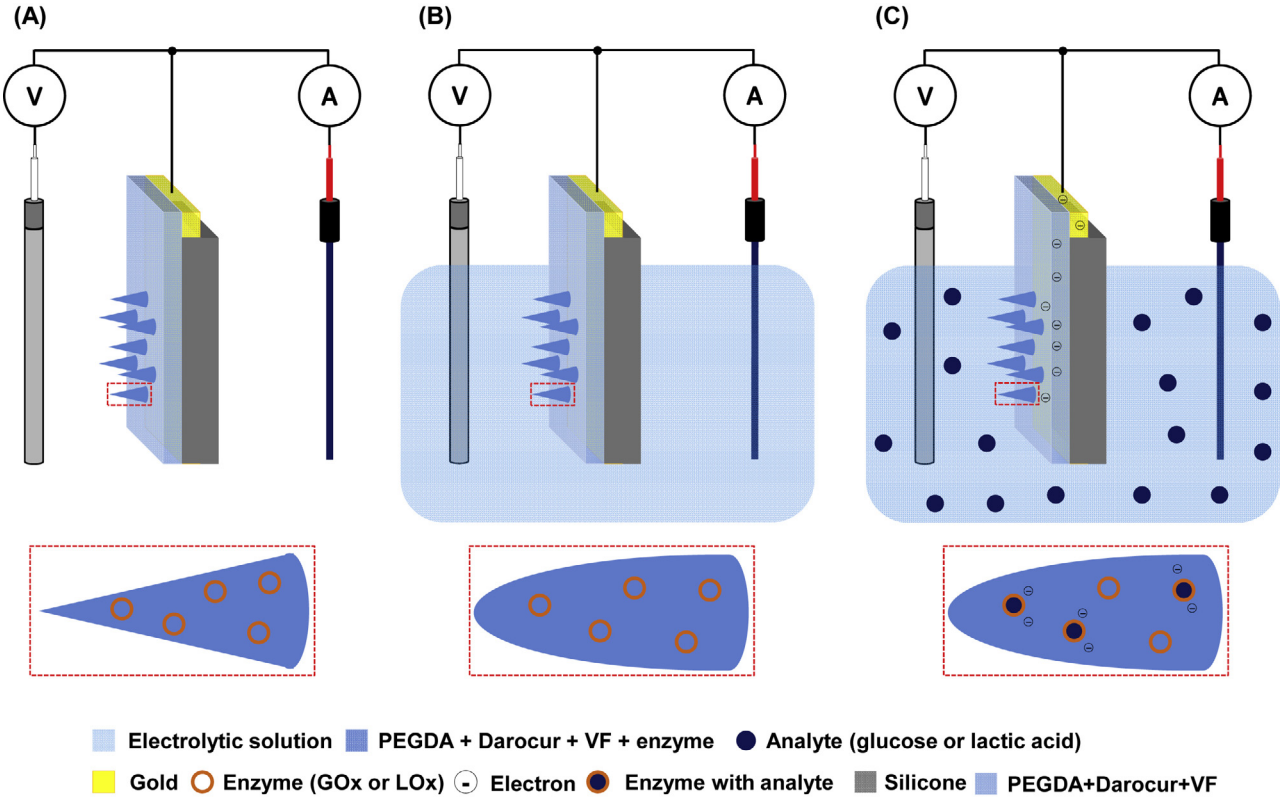

Fig. 4. Sketch of working concept (not in scale). (A) Sketch of the three-electrode system: the MN based enzymatic electrode is the working electrode; a saturated silver chloride electrode (Ag/AgCl) is the reference electrode; a platinum wire is the auxiliary electrode. (B) When the system is soaked in the electrolytic solution, the MNs swell, by allowing the penetration of the solution and its small solutes inside the polymeric matrix, whereas larger molecules as the enzymes (GOx or LOx) are locked in. (C) The analytes interact with the enzymes, resulting in generation of electrons.

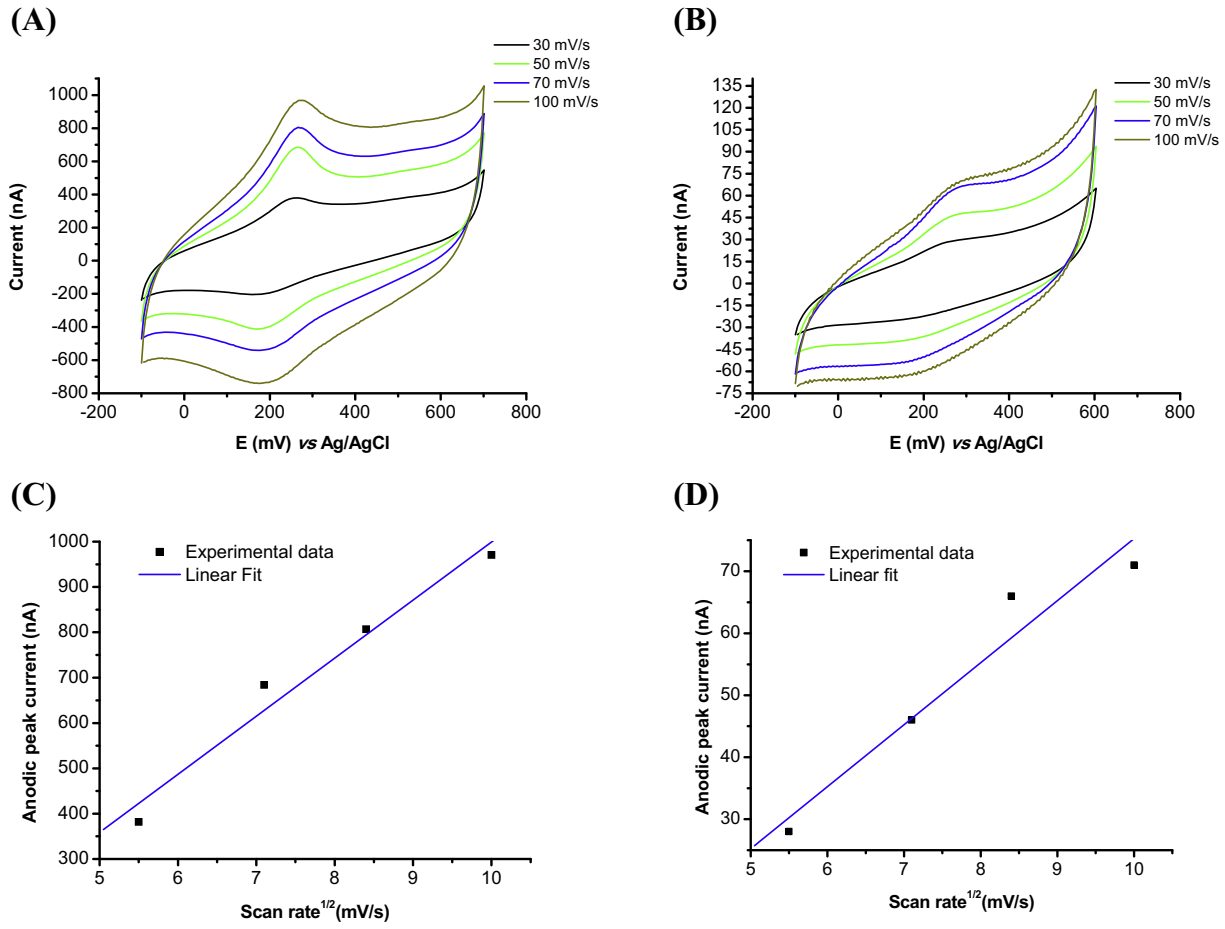

Fig. 5. CV curves characterizing the redox activity of VF in presence of GOx (A) and LOx (B) in the MN polymeric matrix at several scan rates. Anodic current peak values for VF with GOx (C) and LOx (D) show a linear relationship with square root of scan rates.

incorporated into the polymeric matrix, by means of CV at different scan rates (see Fig. 5).

As shown in Fig. 5(A) and (B), the device showed anodic and cathodic peaks at about 300 and 200 mV, respectively, for both enzymes. These values were very close to typical peaks values of free VF [21]. This result confirmed that the polymeric matrix did not interfere with the activity of the redox mediator contained in it. Fig. 5(A) and (B) also highlighted that peak positions were unchanged as a function of scan rate and that reverse to forward peak current ratio was close to unity for both enzymes. Moreover, Fig. 5(C) and (D) demonstrated that the anodic peaks current increased proportionally to the scan rate ($R^2 = 0.95$ and $R^2 = 0.91$ respectively, R^2 being the coefficient of linear regression) for both enzymes. These results suggested that reversible redox process occurred in the MNs based enzymatic electrodes.

CA quantified the biosensor responses at several concentrations of glucose and lactic acid, in concentration ranges of interest for the detection in human interstitial fluid [22–25]. Fig. 6(A) and (B) report the current-time relationship of the two devices at several concentrations of glucose and lactic acid, respectively. Fig. 6(C) and (D) illustrate the calibration curves for glucose and lactic acid, respectively: for each analyte concentration, the stationary current value was considered. The response of the biosensor to glucose was linear (R^2 value of 0.93) with a sensitivity of 18 ± 3 nA mM^{-1} in the range from 0 to 4 mM (inset Fig. 6(C)). The response of the other device to lactic acid was linear (R^2 value of 0.98) with a sensitivity of 3.5 ± 0.2 nA mM^{-1} in the range from 0 to 1 mM (inset Fig. 6(D)). The present biosensor has been patented [26].

Response times were of the order of minutes, and it depended on polymer nanostructure: penetration of solution in the MN matrix was diffusion-driven and faster signal generation can be obtained by tuning its porosity. By using a PEGDA with average molecular weight greater than 250, with longer polymeric chains and wider mesh, should promote analyte penetration and fasten device response. However, PEGDA molecular weight determined MNs mechanical properties, such as indentation hardness; therefore, a tradeoff between analytes diffusion and penetration of the MNs in human skin without breaking could limit the amount of PEGDA with high molecular weight in the polymeric solution. As reported in Ref. [27], faster response time (30 s) can be obtained in the case of MN array enzymatic based biosensor. In our case, a more complex signal processing, based for example on the derivative of the chronoamperometric signal, would lead to a faster response of the device (of the order of second dozens).

The experimental data in Fig. 6(C) and (D) were fitted with Michaelis-Menten model for enzymatic kinetic behavior of the two devices: $I = (I_{max} \cdot c)/(K_M + c)$, where I is the current response of the biosensor, c is the analyte concentration and K_M is the Michaelis constant. The fits yielded a K_M value of 2.7 ± 0.7 mM ($R^2 = 0.99$) in case of glucose, and 0.45 ± 0.03 mM ($R^2 = 0.99$) in case of lactic acid. The K_M represent a measure of how strong is the biomolecular interaction between the enzyme and the analyte: the obtained low values are related to the high affinity between the enzyme (GOx or LOx) and analyte (glucose or lactic acid), confirming that polymeric matrix allows the penetration of the analyte inside it and a correct working of the enzyme, even if confined in a nanometric space. The

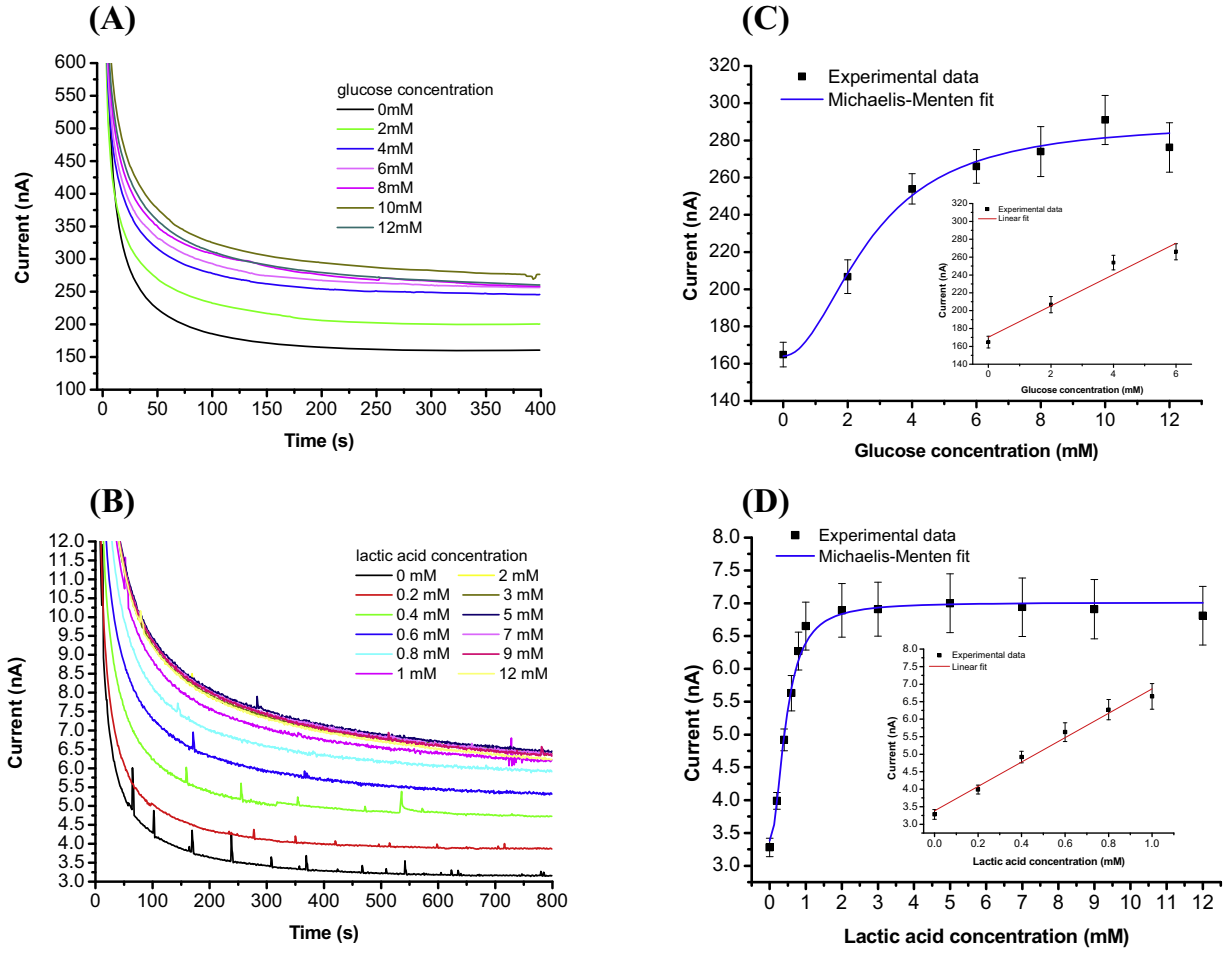

Fig. 6. CA response of the MN based enzymatic electrode at several concentrations of glucose (A) and lactic acid (B). Calibrations curves and fitting lines with Michaelis-Menten model of the MN based enzymatic electrode containing GOx (C) and LOx (D). In the inset, the linear region of the response is underlined and the linear fit is shown.

limits of detection, LoD, have been evaluated as $3\sigma/S$, where σ is the standard deviation of the measured current at 0mMol and S is the sensor sensitivity [27]. For both the two analytes, quantified by respective biosensors, LoD is about 1 μM.

4. Conclusions

Biosensors based on polymeric MNs enzymatic electrodes have been fabricated by standard photolithography of photocurable mixture made by enzymes (GOx/LOx), photoinitiator (Darocur©) and redox mediator (VF). The device has been used to quantify glucose and lactic acid in PBS solutions with concentrations of analytes in the mM range. Good sensitivities, of the order of nA mM^{-1} and limit of detection, 1 μM, have been obtained. Due to peculiar mechanical and electrical properties, the biosensors could be used as wearable patch for continuous monitoring of glucose in diabetes patients blood or for sensing the acid lactic levels in athletes with minimum invasiveness. The innovative technology of polymeric MNs enzymatic working electrodes has been patented [22].

Acknowledgments

This work was partially supported by Italian National Operative Program FUZI (00029_3148467) of IMAST Scarl. The Authors gratefully acknowledge Prof. A. Lombardi of University of Naples Federico II and her team, for electrochemical characterizations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.05.156.

References

[1] J. Wang, Electrochemical glucose biosensors, Chem. Rev. 108 (2) (2008) 814–825.
[2] R.A. Gatenby, R.J. Gillies, Why do cancers have high aerobic glycolysis? Nat. Rev. Cancer 4 (11) (2004) 891–899.
[3] B.M. Spiegelman, J.S. Flier, Obesity and the regulation of energy balance, Cell 104 (4) (2001) 531–543.
[4] A. Vidal-Puig, S. O'Rahilly, Metabolism: controlling the glucose factory, Nature 413 (6852) (2001) 125–126.
[5] N. Nikolaus, B. Strehlitz, Amperometric lactate biosensors and their application in (sports) medicine, for life quality and wellbeing, Microchim. Acta 160 (1–2) (2008) 15–55.
[6] J. Yan, V.A. Pedrosa, A.L. Simonian, A. Revzin, Immobilizing enzymes onto electrode arrays by hydrogel photolithography to fabricate multi-analyte electrochemical biosensors, ACS Appl. Mater. Interfaces 2 (3) (2010) 748–755.
[7] P. Dardano, A. Caliò, V. Di Palma, M.F. Bevilacqua, A. Di Matteo, L. De Stefano, A photolithographic approach to polymeric microneedles array fabrication, Materials 8 (12) (2015) 8661–8673.
[8] J.S. Kochhar, et al., microneedle array fabrication off a photomask to deliver collagen through skin, Pharm. Res. 31 (7) (2014) 1724–1734.
[9] P. Dardano, A. Caliò, J. Politi, I. Rea, I. Rendina, L. De Stefano, Optically monitored drug delivery patch based on porous silicon and polymer microneedles, Biomed. Opt. Express 7 (5) (2016) 1645–1655.
[10] L. Ventrelli, L.M. Strambini, G. Barillaro, Microneedles for transdermal biosensing: current picture and future direction, Adv. Healthcare Mater. (2015), http://dx.doi.org/10.1002/adhm.201500450.
[11] L.M. Strambini, A. Longo, A. Diligenti, G. Barillaro, Lab Chip 12 (2012) 3370.

[12] M.W. Ashraf, S. Tayyaba, N. Afzulpurkar, Micro electromechanical systems (MEMS) based microfluidic devices for biomedical applications, Int. J. Mol. Sci. 12 (6) (2011) 3648–3704.
[13] S. Henry, D.V. McAllister, M.G. Allen, M.R. Prausnitz, Microfabricated microneedles: a novel approach to transdermal drug delivery, J. Pharm. Sci. 87 (8) (1998) 922–925.
[14] P.R. Miller, S.A. Skoog, T.L. Edwards, D.M. Lopez, D.R. Wheeler, D.C. Arango, X. Xiao, S.M. Brozik, J. Wang, R. Polsky, R.J. Narayan, Multiplexed microneedle-based biosensor array for characterization of metabolic acidosis, Talanta 88 (2012) 739–742.
[15] G. Valdés-Ramírez, J.R. Windmiller, J.C. Claussen, A.G. Martinez, F. Kuralay, M. Zhou, N. Zhou, R. Polsky, P.R. Miller, R. Narayan, J. Wang, Multiplexed and switchable release of distinct fluids from microneedle platforms via conducting polymer nanoactuators for potential drug delivery, Sens. Actuators B 161 (1) (2012) 1018–1024.
[16] S. Kaushik, A.H. Hord, D.D. Denson, D.V. McAllister, S. Smitra, M.G. Allen, M.R. Prausnitz, Lack of pain associated with microfabricated microneedles, Anesth. Analg. 92 (2) (2001) 502–504.
[17] H.T. Hammel, Evolving ideas about osmosis and capillary fluid exchange, FASEB J. 13 (2) (1999) 213–231.
[18] X. Hong, Z. Wu, L. Chen, F. Wu, L. Wei, W. Yuan, Hydrogel microneedle arrays for transdermal drug delivery, Nano-Micro Lett. 6 (3) (2014) 191–199.
[19] P.R. Miller, R.J. Narayan, R. Polsky, Microneedle-based sensors for medical diagnosis, J. Mater. Chem. B. 4 (2015) 1379–1383.
[20] J.S. Kochhar, W.J. Goh, S.Y. Chan, L. Kang, A simple method of microneedle array fabrication for transdermal drug delivery, Drug Dev. Ind. Pharm. 39 (2) (2013) 299–309.
[21] H. Ju, D. Leech, Electrochemistry of poly (vinylferrocene) formed by direct electrochemical reduction at a glassy carbon electrode, J. Chem. Soc. Faraday Trans. 93 (7) (1997) 1371–1375.
[22] N.Y. Shim, D.A. Bernards, D.J. Macaya, J.A. DeFranco, M. Nikolou, R.M. Owens, G.G. Malliaras, All-plastic electrochemical transistor for glucose sensing using a ferrocene mediator, Sensors 9 (12) (2009) 9896–9902.
[23] A.J. Shum, M. Cowan, I. Lähdesmäki, A. Lingley, B. Otis, B.A. Parviz, Functional Modular Contact Lens, In SPIE NanoScience+ Engineering, International Society for Optics and Photonics, 2009, pp. 73970K–73970K (August).
[24] N. Thomas, I. Lähdesmäki, B.A. Parviz, A contact lens with an integrated lactate sensor, Sens. Actuators B 162 (1) (2012) 128–134.
[25] H. Yao, A.J. Shum, M. Cowan, I. Lähdesmäki, B.A. Parviz, A contact lens with embedded sensor for monitoring tear glucose level, Biosens. Bioelectron. 26 (7) (2011) 3290–3296.
[26] V. Di Palma, M.F. Bevilacqua, A. Di Matteo, P. Dardano, Microneedle array device for biosensor applications US Application Patent 14558779.
[27] L. Strambini, A. Longo, S. Scarano, T. Prescimone, I. Palchetti, M. Minunni, D. Giannessi, G. Barillaro, Biosens. Bioelectron. 66 (2015) 162.

Biographies

Alessandro Caliò received the International PhD in Novel Technologies for Materials, Sensors and Imaging from the University of Naples Federico II - PhD School in Industrial Engineering in 2016. The thesis, titled "Fabrication of microdevices for biomedical applications based on lithographic approach", was focused on fabrication, using photolithography and soft lithography, and characterization of microdevices for biomedical applications, in clean room. He conducted scientific research activity on microfluidics and biosensoristics fields for the National Research Council - Institute for Microelectronics and Microsystems. He received the Master's degree in Electronic Engineering from the University of Calabria in 2012. The thesis, titled "Design and fabrication of hybrid bio/non-bio interface for a new class of optical glucose sensors", was focused on design, fabrication and characterization of a biosensor based on porous silicon and proteins for optical detection of glucose, in clean room. During all these periods, he acquired good skills to work in a team and in highly specialized laboratories, using advanced technological tools. He actively participated to several national and international conferences, and he is author or co-author of several scientific publications.

Principia Dardano is responsible for the design, fabrication and optical characterization of optoelectronic silicon devices. She graduated in Physics at the University of Studies of Naples "Federico II" in March 2004 and received his Ph.D. in Fundamental and Applied Physics at the same university in January 2008, with thesis on negative refractive index 2D photonic crystals in silicon. Since December 2004 she has worked at the Institute for Microelectronics and Microsystems (IMM-CNR) in Naples, where she is the head of the photolithographic laboratory since 2006. She is the author and co-author of about scientific articles published on peer-reviewed journals. She holds one US patent, and one Italian patent. She is reviewer for several scientific journals.

Vincenza Di Palma is graduated in Chemistry. She has been working in STMicroelectronics since 2002 in R&D division as process and technology Engineer. Her activity deals with Silicon and plastic surfaces chemical functionalization, material deposition process, photolithographic technique and chemical-physical characterization. She worked two years at University of Studies of Naples "Federico II" on liquid-crystalline polymers and flame retardant additives for textiles. During this period she performed as lecturer on contract in Chemistry.She is co-author of 4 scientific articles and 8 European and American patents.

Maria Fortuna Bevilacqua works in STMicroelectronics since 2002. She is currently process and technology Engineer for R&D area with special attention to new materials for the elecronics. She graduated in Physics on March 2001 and she got her Ph.D. in Novel Technologies for Materials, Sensors and Imaging on December 2005 at the University of Naples "Federico II" with a thesis of new synthetic strategies for controlled carbon nanotubes growth. Her activity is focused on physical deposition thechniques, material surface treatments, structural and morphological characterizations, photolithografic processes and basic electrical characterization of inorganic and organic materials. She is co-author of 9 scientific papers on peer-reviewed journals and 8 European and US patents.

Andrea Di Matteo works in STMicroelectronics from July 2004. His current position is the R&D Manager. He got his degree in chemistry in 1995 and a Ph.D. in theoretical chemistry. His scientific formation has been further improved at the Ecole Normale Superiore de Paris (ENS), University of Padova and the Centre Energie Atomique, CEA Grenoble France. His main research interests include the Molecular Magnetism, Dielectric Properties, new Emerging Technology Areas for Heterogeneous Integration, Polymer and Printed Electronics, advanced Bio-systems for Disposable Device, as well as gas sensor and multiscale modeling.

Helena Iuele graduated cum laude in Analytical Chemistry at "La Sapienza" University of Rome in 2013. She won a training project scholarship to become a researcher specialized in polymeric materials with integrate functionality at IMAST scarl of Naples, Italy. Actually she is a Ph.D. scholarship candidate in Chemistry at the "University of Waikato" Hamilton, New Zealand. Her research interests include synthesis and analytical characterization of nanostructured materials and environmental monitoring using active or passive sampling strategies coupled with chromatographic techniques. She is author of a poster awarded in 2014 at the "VI Young Chemists' Conference" held in Rome and she holds one American Patent.

Luca De Stefano graduated cum laude in Physics at University of Naples "Federico II" in 1992 and received the Ph.D. in Physics in 1996. He is senior scientist at the Institute for Microelectronic and Microsystems of National Research Council in Naples, where he heads a small research group in the fields of biophotonics and optical microsystems for biochemical sensing. He presented his work to more than 170 national and international conferences, many of which he has been invited to. He is author or co-author of more than 115 scientific articles published on peer reviewed journals, more than 70 conference proceedings, and eight books. He holds one European patent, and eight Italian patents. He is reviewer for many high impact factors scientific journals.