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Biodegradable Mats for the Design of Bifunctional Biosensors for Glucose Detection in Urine †

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Abstract: We introduce a bifunctional architecture to construct biosensors on solution-blow spinning fiber mats of polylactic acid (PLA) and polyethylene glycol (PEG). PLA/PEG mats acted as substrate for printing electrodes and as matrix to immobilize glucose oxidase (GOx). Prussian Blue nanoparticles (PB) decorated working electrodes to detect H_2O_2 from enzymatic catalysis at a low applied potential (0 V vs Ag/AgCl) with detection limit of 0.197 mM. The inexpensive bifunctional device (<US $0.25 per unit) exhibited a rapid response, long lifetime and performance similiar to the standard method for glucose monitoring in human urine samples. The PLA/PEG mats are sustainable alternative for biosensing and wearable applications.

Keywords: glucose; biosensor; screen-printed electrodes; Prussian blue; urine; non-invasive; PLA; sustainable substrates

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1. Introduction

Flexible (bio)sensors are a new generation of electronic devices, that since the glucose biosensor invention in 1962, have attracted much attention [1] due to the need of monitoring relevant biomarkers of status health using smartphones, laptops, and smartwatches [2]. Electrochemical (bio)sensors are now becoming popular due to the several efforts made in plenty of applications including forensic analyses [3], wearable sensors [4], potentiometric [5], clinical analyses, personalized medicine [6], environmental [7] and the food industry [8].

Diabetes mellitus is a severe disease reaching about 422 million people in the world and is still a cause of death [9]. The commercial glucometer used to detect glucose levels in blood samples is an indispensable tool for diabetes management [1,10]. Nowadays, glucose biosensors are pursuing less invasive tests for clinical analyses using biological fluids including saliva [11], tears [12], urine [13], and sweat [14] samples, and with different approaches in the sensing layer to improve analytical performance [1,10]. The electrochemical (bio)sensors existent has been made onto plastic support e.g., polyimide, polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyurethane (PU), and polydimethylsiloxane (PDMS) [15,16]. The massive use of (bio)sensors can generate an expressive

amount of electronic waste because these materials are not easily degradable in the environment [16]. Still, there is an urgent demand for alternative materials for the design of eco-friendly, biodegradable and sustainable flexible bioelectronics [16].

Herein, we proposed a bifunctional support of polylactic acid (PLA) and polyethylene glycol (PEG) prepared by the solution-blow spinning technique to design an electrochemical biosensor. The PLA/PEG nanofibers are degradable, free of waste, low-cost, and considered a sustainable material, making them promising for electrochemical biosensors projection. The substrate was namely bifunctional because they were employed as support for screen-printed carbon electrodes (SPE) and as a matrix for covalent immobilization of glucose oxidase (GOx). The GOx was incorporated directly on PLA/PEG surface and was responsible for hydrogen peroxide production which is detected on the SPCE surface. Prussian Blue nanoparticles (PB) were electrodeposited on the SPCE surface to decrease the cathodic potential for hydrogen peroxide detection allowing to perform the chronoamperometric measurements with a low applied potential of 0 V vs. Ag/AgCl directly in undiluted human urine.

2. Materials and Methods

2.1. Preparation of PLA/PEG Mats

The PLA/PEG mats (mol. wt., 76.000:8.000) were made using the solution-blow spinning technique. The specific protocol and setup used for PLA/PEG preparation can be found in Paschoalin et al 2022 [17] and Gomes et al 2023 [9].

2.2. Deposition of Electrodes on PLA/PEG Mats

The PLA/PEG sensor was fabricated using screen-printed technology illustrated in Figure 1A,B [18]. The carbon paste (ELETRODAG 423SS E&C) was deposited in a polyester screen (77-mesh) followed by transfer to PLA/PEG mats using a polyurethane squeegee (75 sh) as indicated in Figure 1A,B. The printed device in Figure 1Ci was cured at 90 °C for 30 minutes followed by the manual painting of the pseudo-reference electrode using a commercial silver/silver chloride paste (TICON®, (CODE: 90701)) and cured at 90 °C for 30 minutes. The PLA/PEG sensor contain a reference (RE), counter (CE) and working electrodes (WE) being the thickness of the device about 400 μm (see Figure 1Cii). The WE have a geometric area of 0.1256 cm^2.

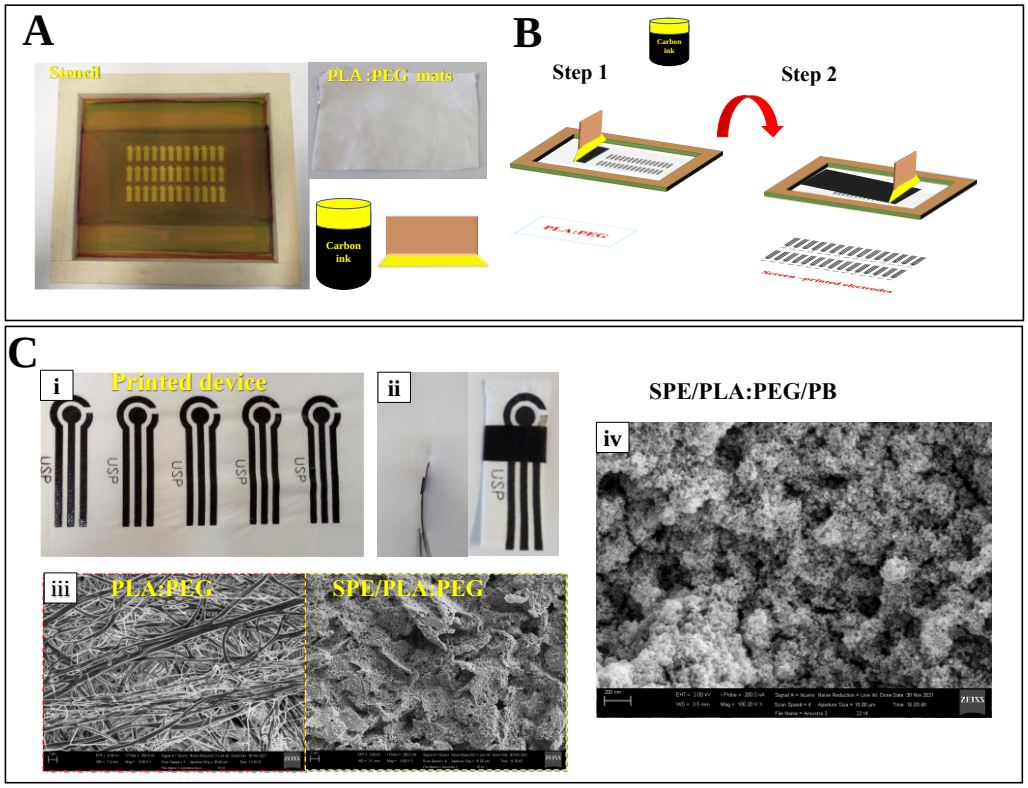

Figure 1. Representation of the materials used for the fabrication of the device in (**A**). Scheme of the screen printing protocol in (**B**). Photo of the PLA/PEG electrodes in (**C**)i and (**C**)ii. SEM-FEG images of the PLA/PEG mat and bare surface of WE in (**C**)iii. Images of WE modified with PB nanoparticles in Civ.

2.3. Electrochemical Deposition of Prussian Blue Nanoparticles (PLA: PEGSPE//PB)

Before use, as prepared PLA/PEG sensors were submitted to an electrochemical pre-treatment as detailed in our previous work [18,19]. The deposition of the PB nanoparticles on PLA/PEG/SPE surface was made using cyclic voltammetry experiments in a potential range from −0.2 to 1V (100 mV s^{-1}) for 10 cycles using 200 µL of the solution containing 1.5 mM $FeCl_3$ + 1.5 mM $K_3[Fe(CN)_6]$ + 1 mM CTAB (prepared in 0.1 M KCl, pH = 1.66) [9,20].

2.4. Glucose Oxidase (GOx) Immobilization

The acid carboxylic group onto PLA/PEG/SPE/PB was activated by drop-cast 40 µL of EDC/NHS (0.4/0.1 M) solution and kept for 30 minutes [21]. The unreacted EDC/NHS was removed from the PLA/PEG surface by rinsing the device with ultra-pure water and dried with nitrogen air [9]. Then, 20 µL of 10 mg mL^{-1} glucose oxidase solution was drop-casted on the PLA/PEG mats (not on ink surface) and the biosensor was kept in the refrigerator overnight (4 °C) [9]. Before use, the PLA/PEG biosensor was immersed in BR buffer solution (pH = 6.0) to remove glucose oxidase weakly adsorbed at the PLA/PEG surface [9]. Figure 2 summarizes the fabrication principle of the bifunctional surface.

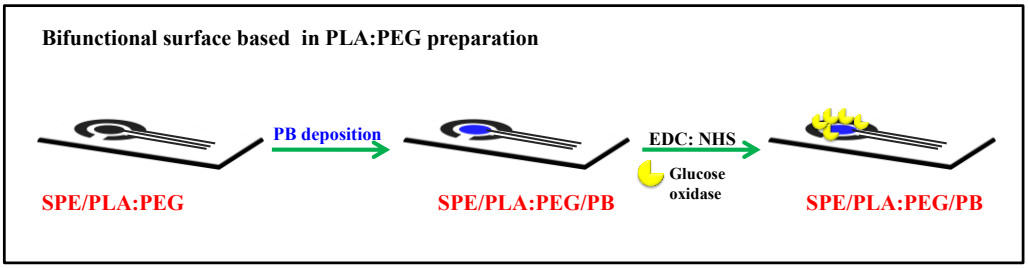

Figure 2. Fabrication principle of the bifunctional surface.

2.5. Electrochemical Measurements

All electrochemical measurements were conducted in an electrochemical cell containing 20 mL of artificial urine (pH = 6.0 containing 0.1 M of KCl solution) as a supporting electrolyte [9]. Chronoamperometric measurements of glucose detection were made by applying 0 V (versus Ag/AgCl) for 30 seconds with an incubation time of 60 seconds between the analyses [9]. All the experiments using undiluted human urine samples were conducted in strict agreement with the ethical committee of Brazil (project# 54796721.8.0000.5422) [9].

3. Results

Figure 1Ciii displayed the SEM-FEG images of the PLA/PEG mats composed by fibers with a diameter of 351 (±118) nm. The working electrode image in Figure 1Ciii shows a well-distributed ink layer composed by graphitic and carbon black nanoparticles on PLA/PEG mats indicating a successful printing process. Figure 1Civ depicted small nanoparticles of Prussian blue (PB) covering homogeneously WE surface.

Figure 3A indicates the working principle of the bifunctional surface. Glucose oxidase enzyme catalytically converts glucose in presence of molecular oxygen into gluconic acid and hydrogen peroxide. PB electrodeposited on WE are reduced at 0.0 V to PW form [22,23]. The hydrogen peroxide produced by the enzymatic reaction is reduced by PW form [24,25]. Figures 3B and 3C show the chronoamperometric response obtained at 0 V for increasing concentrations of glucose in artificial urine (pH = 6.0) using PLA/PEG/SPE/PB (without GOx enzyme) and with the biosensor, respectively. The control experiments using PLA/PEG/SPE/PB reveals no increase in the current signal with successive glucose additions (Figure 3B). On the other hand, when the GOx enzyme was attached on PLA/PEG fibers there was a current increment with a glucose concentration between 0 and 5.5 mM indicating the catalytic production of hydrogen peroxide and the electrochemical reduction using the biosensor. The current response using biosensor was proportional to glucose concentration, resulting in two linear calibrations curves expressed as $I\ (A) = -5.93 \times 10^{-9} + 0.003\ C_{glucose}\ (M)$, $R^2 = 0.998$ and $I\ (A) = 3.91 \times 10^{-6} + 0.0018\ C_{glucose}\ (M)$, $R^2 = 0.999$. The detection limit was estimated with Miller & Miller [26,27] method yielding a value of 0.197 mM. Our approach opens a new scenario to explore the properties and applications of the bifunctional surfaces based on sustainable PLA/PEG mats.

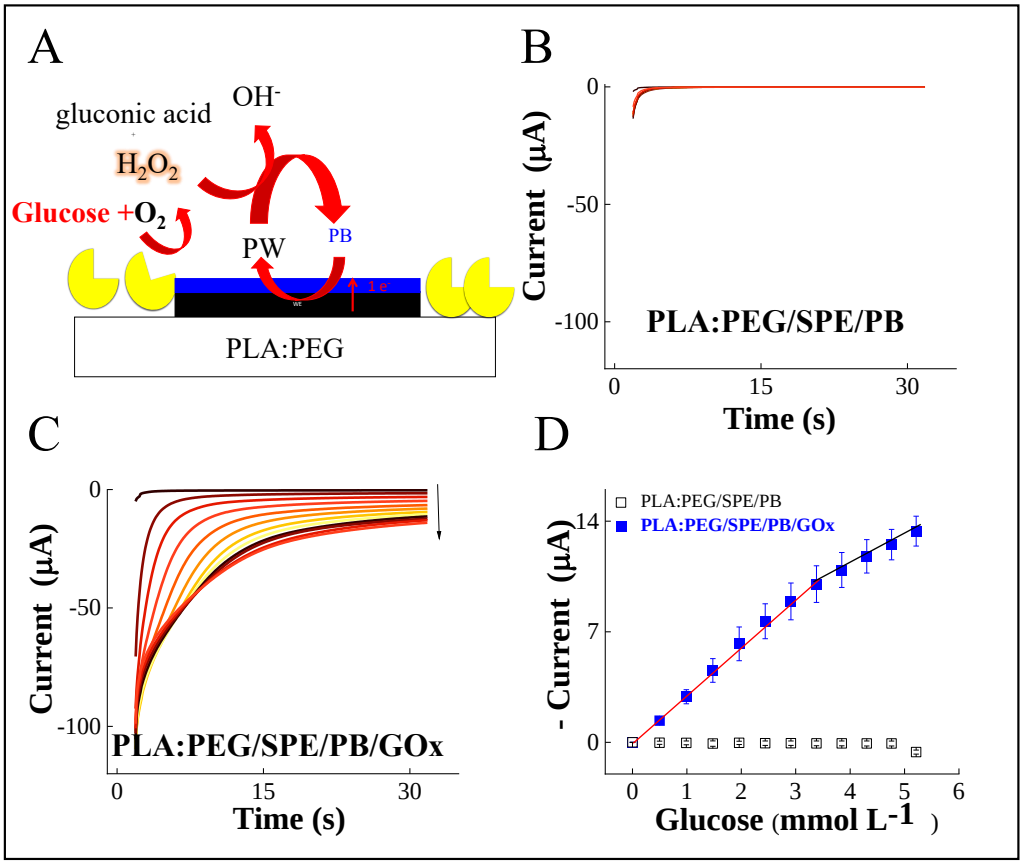

Figure 3. (**A)** Schematic representation of the operational principle of bifunctional surface based on PLA/PEG mat. Chronoamperograms obtained for increasing glucose concentration between 0.5 and 5.24 mM using PLA/PEG/SPE/PB (without GOx enzyme) in (**B)** and biosensor in (**C)**. (**D)** The resulting calibration curve from the surfaces. All experiments were made in artificial urine (pH = 6.0, 0.1 M of KCl) with E_{appl} = 0 V (v.s Ag/AgCl) for 30 seconds.

Real Sample Analysis, Interference and Stability

The glucose determination in undiluted human urine was made using the standard addition method. Figure 4A shows the glucose concentration found using the biosensor and the spectrophotometric gold standard method. Both methods estimated concentrations of glucose close to 0.9 mM indicating good precision and agreement between the analytical methods. The selectivity test of biosensor consisted in recorded the chronoamperometry response of 2 mM glucose solution followed by the individual addition of uric acid (0.22 mM), lactic acid (0.22 mM), paracetamol (40 µM), dopamine (40 µM) and ascorbic acid (40 µM). The compounds exhibited a slight change in the current signal (< 8%) as indicated in Figure 4B. The high specificity achieved by biosensor was owing to the PB transducer allowing to operate at a low potential of 0 V. The storage stability of the biosensor shown in Figure 4C was evaluated over 60 days yielding a standard deviation of 12% (n = 8) for the current values. All those outcomes indicate the potential of the bifunctional device for glucose monitoring using noninvasive method.

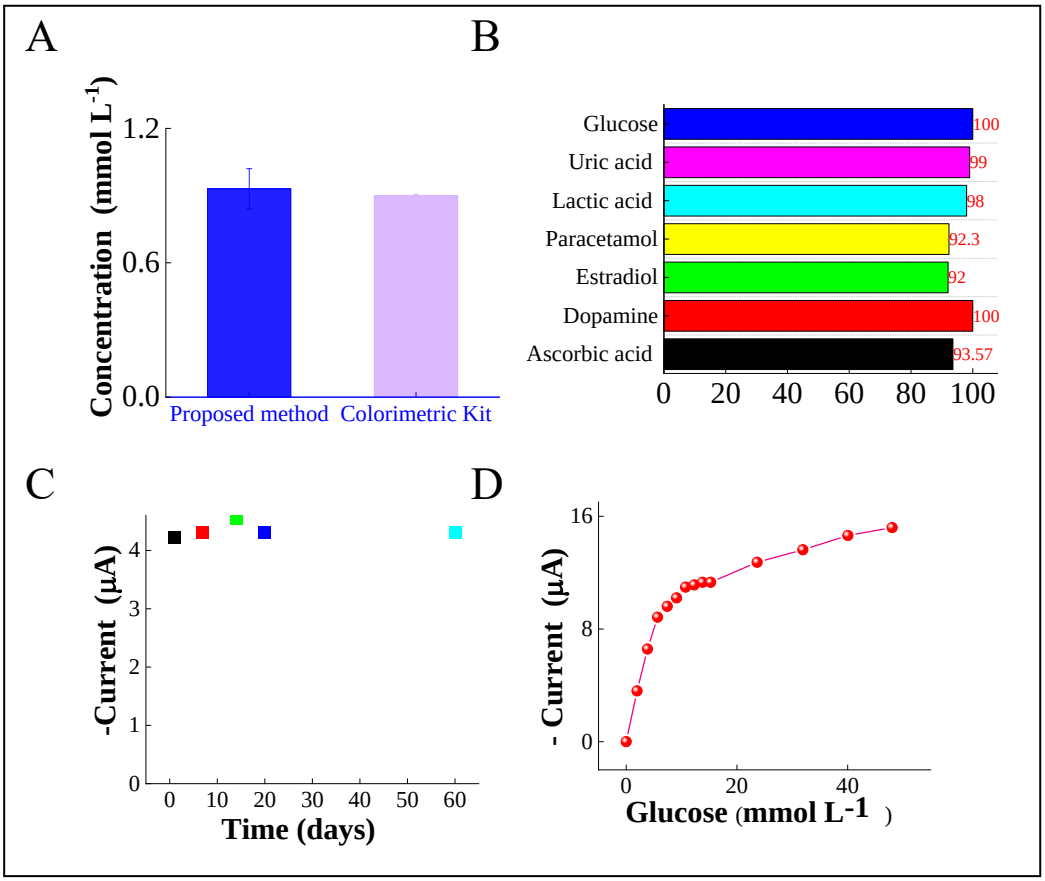

Figure 4. Bar plot of glucose determination in undiluted human urine using the biosensor and standard method in (**A**). Interference percentage in (**B**). Stability study during 60 days in (**C**). Graphic for determination of enzymatic constant in (**D**).

4. Conclusions

We offered a bifunctional surface based on PLA/PEG mats used as support for printing flexible sensors and as matrix for glucose oxidase immobilization. The design allowed the modification of WE with PB nanoparticles to be free from biofouling effects and improve the efficiency of the biodevice for detecting hydrogen peroxide at low potential (0 V vs Ag/AgCl). The PLA/PEG biosensor showed a LOD of 197 µM and high sensitivity of 0.024 A M^{-1} cm^{-2} for selective monitoring of glucose in undiluted urine samples with long-term stability over 60 days. PLA/PEG mats are promising support for designing eco-friendly, sustainable and biodegradable electronic devices being a green option to reduce the use of petroleum-based plastic materials.

Author Contributions: N.O.G.: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Resources; Writing—Original Draft; Writing—Review & Editing. R.T.P.: Conceptualization; Formal analysis; Investigation; Resources. A.R.S.: Formal analysis; Investigation; Resources; Writing—Review & Editing. S.B.: Investigation; Resources; Writing—Review & Editing. C.S.F.: Resources; Writing—Review & Editing. L.H.C.M.: Resources; Writing—Review & Editing; Funding acquisition. S.A.S.M.: Resources; Writing—Review & Editing; Funding acquisition. O. N. O. Jr.: Resources; Writing—Review & Editing. P.A.R.P.: Conceptualization; Methodology; Resources; Writing—Original Draft; Writing—Review & Editing; Visualization; Supervision; Project administration; Funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of São Paulo University (protocol code 54796721.8.0000.5422 and date of approval 16 of January of 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper" if applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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