Enzyme and Microbial Technology 126 (2019) 24–31

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Cells-on-nanofibers: Effect of polyethyleneimine on hydrophobicity of poly-Ɛ-caprolacton electrospun nanofibers and immobilization of bacteria

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ARTICLE INFO

Keywords:
Electrospun nanofiber
Biofunctional surface
Whole-cell biosensor
G. oxydans
Covalent immobilization

ABSTRACT

Among other synthetic polymers, poly-Ɛ-caprolacton (PCL) nanofibers are one of the most popular ones, especially in tissue engineering application due to its distinct mechanical and chemical properties. However, in some cases, lacking functional group on polymer structure obstructs the covalent modification of the PCL nanofibers for the aim. Herein, polyethyleneimine (PEI) was blended with PCL polymer to provide functional amino groups on the surface of the nanofiber mat. PCL-PEI nanofiber was successfully constructed and preparation parameters were optimized. Scanning electron microscopy (SEM) and contact angle measurements were carried out to characterize the PCL-PEI nanofiber. After characterization, *Gluconobacter oxydans* was immobilized on the surface by the help of glutaraldehyde chemistry. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were carried out to prove the success of surface modification. In addition, scanning electron microscopy images were also taken after the immobilization of *G. oxydans* on PCL-PEI nanofiber mat. For the first time in this study, one microorganism was immobilized onto the electrospun nanofiber mat by covalent modification. In conclusion, PCL-PEI/*G. oxydans* whole-cell biosensor was tested for sensing of glucose as a model analyte.

1. Introduction

Microbial whole-cell biosensor is a research field which comprise different kinds of discipline such as microbiology, electrochemistry, material science, biochemistry and technology [1]. Recently, microbial whole-cell biosensors have gained attractive attention due to expansion related to the interaction of different disciplines and the demands of biotechnological processes. Electrochemical microbial biosensors use microorganisms as a bio-recognition component to detect some compounds. Although purified enzymes are widely used in the field of biosensing, purification process is time-consuming and expensive. Moreover, in some cases they are less stable and more sensitive to environmental changes [2]. However, enzymes within microorganism preserve their nature and large number of microorganisms can be produced and manipulated by cell-culturing methods to construct biosensor platforms [3]. Various kinds of biomass such as *Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Escherichia coli, Saccharomyces cerevisiae* have been used as a recognition element of whole-cell biosensors [4–8]. Among them, *Gluconobacter oxydans (G. oxydans)* is a special strain. It is a gram negative, obligate aerobe which is widely used in variety of biotechnological application and amperometric biosensors (Gluconosensors) [9,10]. Because, most of the important reactions can be catalyzed by oxidative enzymes (PQQ-dependent dehydrogenases) which is located in periplasm of *G. oxydans* [11]. Due to the location of these enzymes, there is also no need to transport substrates into the cells that can affects response time of microbial sensor positively [12]. To construct prospering Gluconosensor, it is crucial to maintain the active metabolism of *G. oxydans* extended period of time. Different types of organic, inorganic and hybrid materials as an immobilization matrix have been utilized to modify the transducers by *G. oxydans* cells [13,14].

Electrospun nanofibers are good alternatives for the immobilization of microbial cells. For example, Uyar et al. immobilized *Lysinibacillus* sp. NOSK bacteria in cyclodextrin (CD) nanofibers and the potential application of this bacteria/CD biocomposite in bioremediation of heavy metals (Ni(II) and Cr(VI) ions) and reactive dye from aqueous solution was systematically investigated [15]. Tekinay et al. immobilized *Aeromonas eucrenophila*, *Clavibacter michiganensis* and *Pseudomonas aeruginosa* strains in cellulose acetate nanofibrous web (CA-NFW) for decolorization of methylene blue dye in wastewater treatment [16]. Furthermore, De Gregorio et al. were immobilized *Pantoea agglomerans* ISIB55 and *Burkholderia caribensis* ISIB40 strains in polyvinyl alcohol (PVA) nanofiber to test the application of this web as a soybean seed bioinoculants [17].

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https://doi.org/10.1016/j.enzmictec.2019.03.002
Received 7 December 2018; Received in revised form 23 February 2019; Accepted 2 March 2019
Available online 02 March 2019
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Table 1
Optimization of PCL-PEI nanofibers.

Initial concentration of Polymer Solution (% w/v)	Solvent System (v:v)	Final concentration of polymers (w/v) in mixture (PCL:PEI, v/v ratio)	Comments
10% PCL 10% PEI	Chl:DMF (3:1) Chl:DMF (3:1)	5% PCL, 5% PEI (1:1)	Dissolution of PEI after adding of Chl
10% PCL	Chl:DMF (3:1)	9.5 % PCL, 0.5 % PEI (20:1)	
10% PEI	DMF	9.1 % PCL, 0.9 % PEI (10:1)	
		8.3 % PCL, 1.7 % PEI (5:1)	Non-uniform fibers
		8.0 % PCL, 2.0 % PEI (4:1)	Beaded fibers
		7.5 % PCL, 2.5 % PEI (3:1)	
		6.7 % PCL, 3.3 % PEI (2:1)	
		5.0 % PCL, 5.0 % PEI (1:1)	
10% PCL	THF:DMF (3:1)	8.3 % PCL, 1.7 % PEI (5:1)	Non-uniform fibers
10% PEI	DMF	8.0 % PCL, 2.0 % PEI (4:1)	Bead free and uniform fibers
		5.0 % PCL, 5.0 % PEI (1:1)	
		6.7 % PCL, 3.3 % PEI (2:1)	
		3.3 % PCL, 6.7 % PEI (1:2)	

Poly-Ɛ-caprolacton (PCL) is a hydrophobic, biocompatible polymer which is one of the most favored material for electrospinning application ought to its multifunctional feature such as its biodegradability and biocompatibility [18]. Various types of polymers such as chitosan, polyethylene oxide, poly(*N*-vinyl-2-pyrrolidone) etc. have been added to PCL nanofibers [19–22]. Polyethyleneimine (PEI) is an alternative polymer to provide the functional group for the covalent immobilization of biological macromolecules. PEI is a water-soluble polyelectrolyte that has enormous amount of protonated amino groups below pH value of 10 [23]. With the help of extensive positive charges on polymer chain, PEI is known as a promising polymer to support cell attachment in tissue engineering applications. In literature, PCL and PEI composed nanofibers have been used to examine as a scaffold for cell attachment and proliferation of human umbilical vein endothelial cells (HUVECs) and NIH 3T3 fibroblasts cell line until now [24,25]. Herein, PEI was blended with PCL to enable the covalent immobilization of microbial cells on nanofiber surface by providing free amine groups. According to our knowledge, there was no study comprising immobilization of microorganism via covalent bonds on nanofiber surface to construct amperometric microbial biosensor until now.

In this study, PCL-PEI nanofiber mat was formed successfully, preparation conditions were optimized and the PCL-PEI electrospun nanofiber was characterized by scanning electron microscopy (SEM) and contact angle measurements. Then, *G. oxydans* cells were immobilized via glutaraldehyde chemistry. To show the success of immobilization, CV and DPV measurements were carried out and SEM images were taken. Finally, the PCL-PEI/*G. oxydans* microbial whole-cell biosensor was calibrated for glucose to test immobilization success on electrospun nanofibers.

2. Experimental

2.1. Materials

Poly-Ɛ-caprolacton (PCL) (Mn; 80.000), polyethyleneimine (PEI) (50% w/v), tetrahydrofuran (THF; ≥99%), dimethylformamide (DMF; ≥99%), glutaraldehyde (GA; 25%), yeast extract, KCl and glucose were purchased from Sigma Aldrich. Artificial human urine was prepared by mixing 0.65 g/L $CaCl_2.2H_2O$, 0.65 g/L $MgCl_2.6H_2O$, 4.60 g/L NaCl, 2.30 g/L Na_2SO_4, 0.65 g/L $Na_3citrate.2H_2O$, 0.02 g/L Na_2-$(COOH)_2$, 4.20 g/L KH_2PO_4, 1.60 g/L KCl, 1.00 g/L NH_4Cl, 25.00 g/L NH_2CONH_2 (urea) and 1.10 g/L $C_4H_7N_3O$ (creatinine) [26]. Artificial human saliva was prepared by mixing 0.126 g/L NaCl, 0.964 g/L KCl, 0.189 g/L KSCN, 0.654 g/L KH_2PO_4, 0.200 g/L NH_2CONH_2 (urea), 0.763 g/L $Na_2SO_4.10H_2O$, 0.178 g/L NH_4Cl, 0.228 g/L $CaCl_2.2H_2O$, 0.630 g/L $NaHCO_3$ [27]. Artificial tear (Refresh tears, Allergan) was procured from local pharmacy.

2.2. Apparatus

A NanoWeb Electrospin 103 (MaviTech, Turkey) apparatus was used for nanofiber preparation and syringe pump was from New Era Pump System. Electrochemical measurements were done with PalmSens potentiostat (Palm Instruments Houten, Netherlands) and glassy Carbon Electrode (GC), platinum electrode (BASI, West Lafayette Indiana, ABD), and Ag/AgCl electrode (Metrohm, Swiss) (as a triple electrode system) were used for working, counter and reference electrodes, respectively. Contact angle measurements of ITO (Teknoma Inc., Turkey) surfaces coated by PCL-PEI nanofibers were carried out by Attension Theta Goniometer. Preparation of the cell pellet for immobilization was done by centrifugation (Denley BS400 Sandwich, UK.) For cell growth, New Brunswick Scientific Edison, Innova 40R incubator was used. Monitorization of PCL-PEI and PCL-PEI/*G. oxydans* surfaces was done by SEM (System Zeiss Sigma 300).

2.3. Preparation of electrospun nanofibers

To prepare the polymer solution for electrospinning process, first PCL and PEI polymer solutions were dissolved individually. 10% (w/v) PCL was dissolved in THF under heat treatment at 50 °C for 2 h. After dissolution of PCL, DMF was added in the ratio of 1:1 (v:v) and the solution was mixed. 10% (w/v) PEI was dissolved in DMF and mixed with PCL solution in the ratio of 2:1 (PCL: PEI, v/v). PCL-PEI polymer solution was mixed until the complete dissolution was achieved. Before electrospinning, PCL-PEI solution was filled in syringe fitted with metallic needle within inner diameter of 8.8 mm. The electrospinning process was conducted under sorted parameters; applied voltage; 9.0 kV, flow rate; 1.45 mL/h, needle-collector distance; 9.0 cm. Electrospinning process was carried out at 26 °C and 56% relative humidity. PCL-PEI nanofibers were collected on GC electrode for approximately 5 min to form the immobilization platform for microbial whole-cell biosensor.

2.4. Bacterial cell cultivation

Lyophilized strain of *G. oxydans* (DSM2343) was purchased from German Collection of Microorganisms and Cell Cultures GmbH (DSMZ), dissolved according to instruction in Medium 105 and stored at −80 °C in 50% (w/v) sterile glycerol for further use. During the experiments, the stock bacterial strain was inoculated into yeast extract medium (5 g/L, 50 mL in 250 mL erlenmeyer flask with 0.25 g glucose) and incubated at 26 °C for 18 h with the constant shaking at 175 rpm.

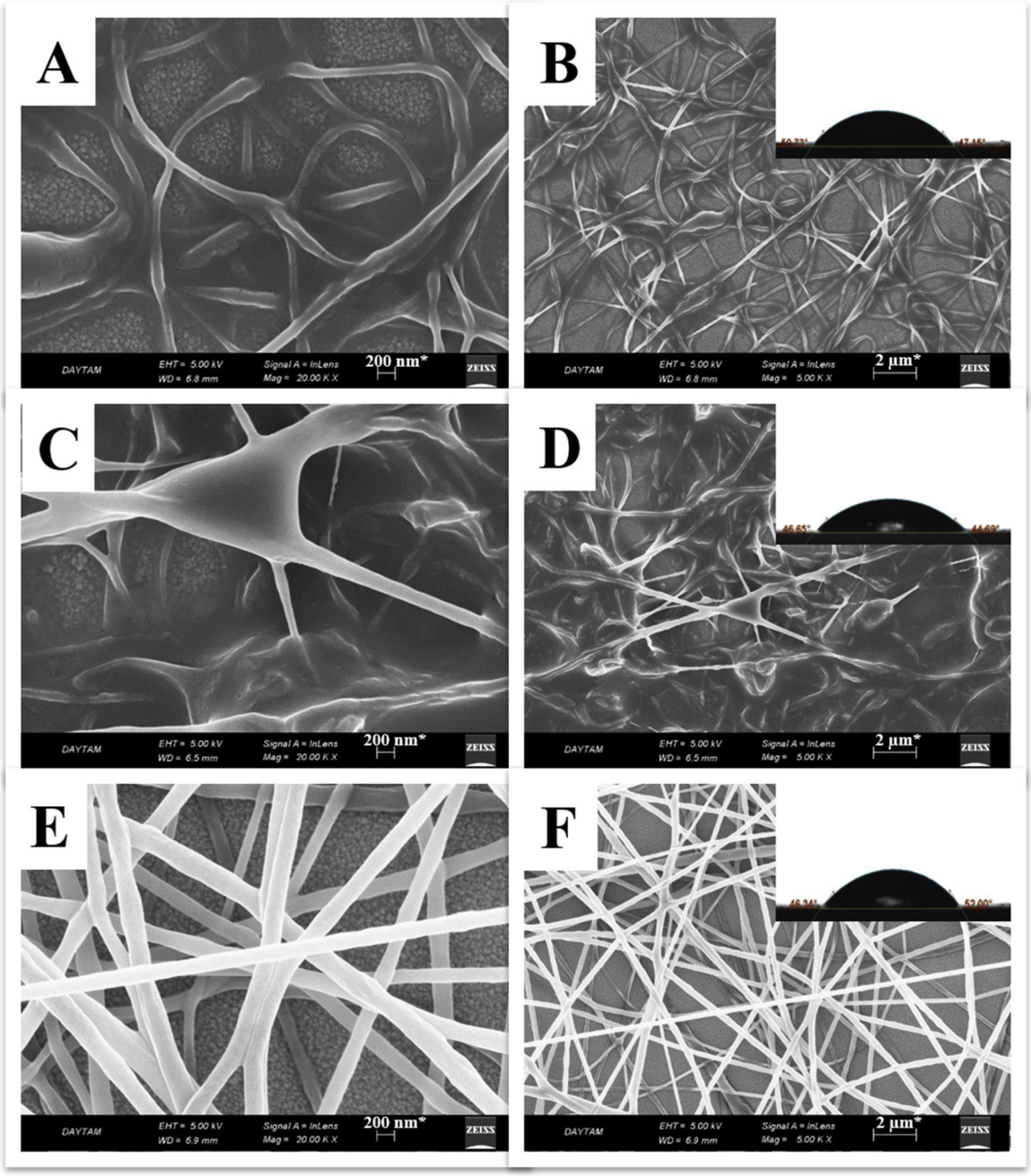

Fig. 1. SEM images of A) PCL-PEI, 1:1 v/v (20 KX); B) PCL-PEI, 1:1 v/v (5 KX); C) PCL-PEI, 1:2v/v (20 KX); D) PCL-PEI, 1:2 v/v (5 KX); E) PCL-PEI, 2:1 v/v (20 KX) and F) PCL-PEI, 2:1 v/v (5 KX) surfaces [inset images show the contact angles of PCL-PEI surfaces].

2.5. Cell immobilization on GC electrode

The optic density of cells was determined by spectrophotometric measurement at 600 nm wavelength. Sterile growth medium was used as a blank. Then the freshly cultured cells were collected by transferring 10 mL of cultivation medium in a falcon tube, centrifuging at 5000 g for 10 min at room temperature, and discarding culture supernatant. Then, the bacterial pellet was re-suspended in pH 5.0, 50 mM Na-Acetate buffer. After resuspension of the pellet in Na-Acetate buffer, cell suspension (8.5×10^8 cell titer) was mixed with glutaraldehyde solution (1.0% in pH 7.0, 50 mM PBS buffer) in the ratio of 1:1 (v/v). 10 µL of the mixture was spread over PCL-PEI/GC surface and left for drying for 1 h at room temperature.

2.6. Electrochemical measurements

All electrochemical measurements (Amperometry; cyclic voltammery, CV and differential pulse voltammetry, DPV) were done with bare GC and PCL-PEI, PCL-PEI/*G. oxydans* coated GC in pH 5.0, 50 mM Na-Acetate buffer at room temperature. Alumina in different particle sizes (0.05 M, 0.1 M, 0.3 M) was used to clean the GC surface and the electrodes were washed with distilled water before using.

All amperometric measurements of PCL-PEI/*G. oxydans* coated GC electrode were done at -0.7 V in pH 5.0, 50 mM Na-Acetate buffer at room temperature under gentle stirring. CV and DPV measurements of bare, PCL-PEI coated and PCL-PEI/*G. oxydans* coated GC electrodes were carried out at the potential rate of -0.4 V to + 0.8 V with the scan rate of 50 mV/s in the presence of 5.0 mM $K_3Fe(CN)_6$ and 0.1 M KCI as

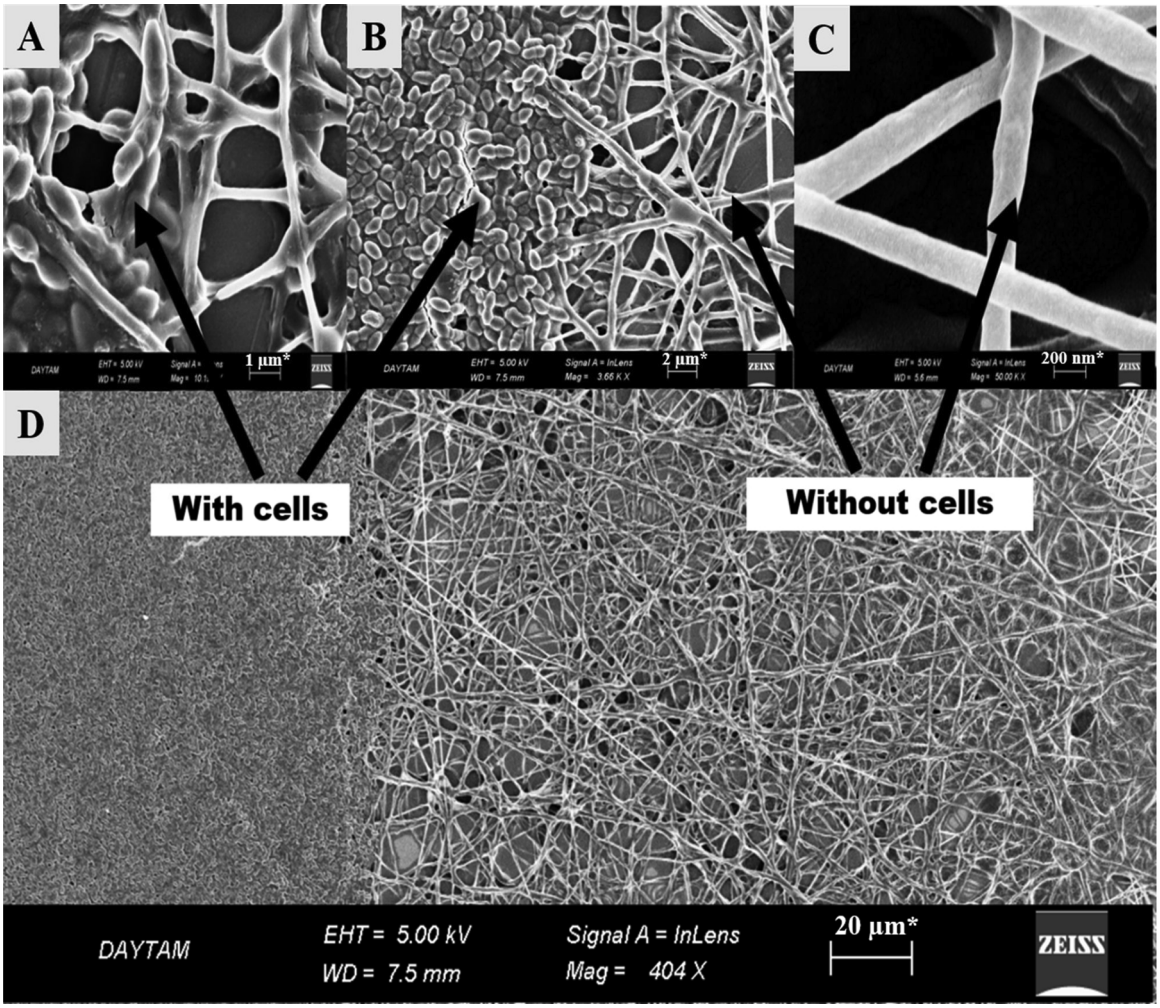

Fig. 2. SEM micrographs of *G. oxydans* immobilized and non-immobilized PCL-PEI nanofibers with different magnifications, A) Immobilized *G. oxydans* on PCL-PEI nanofibers (10 KX) and C) individual PCL-PEI nanofibers (50 KX), D) and B) both *G. oxydans* immobilized (left side of images) and non-immobilized (right side of images) part of PCL-PEI nanofiber surface with the magnification of 3.66 KX and 404 X, respectively.

the auxiliary electrolyte.

GraphPad Prism version 5.03 software (GraphPad Software, San Diego, CA) was used to obtain statistical analysis. Difference between groups was determined using one-way analysis of variance (ANOVA)-Tukey's multiple comparison post-test.

3. Results and discussion

3.1. Optimization of nanofiber preparation conditions

The aliphatic polyester, PCL is a synthetic polymer that has been approved by Food and Drug Administration (FDA). Due to its good electrospinnability, mechanical and chemical properties along with biocompatibility, it is one of the most widely used biomaterial for biological applications recently [28,29]. PEI is a cationic polymer that has an enormous amount of secondary amine group on the molecular structure. Owing to the cationic charge on the surface of the polymer, PEI has been studied frequently as a vector for gene delivery [30]. Beside this, PEI nanofibers were also applied successfully in electrochemical biosensor area [31].

In order to achieve the continuous and precise production of nanofibers, solution and operational parameters should be optimized. Therefore, smooth and bead-free nanofibers with fine diameter can be produced in accordance with the purpose. Regarding optimization, the first step is choosing an appropriate solvent for the electrospinning process. Here, as can be seen in Table 1, the various solvent systems with various PCL and PEI solution ratio (v/v) were tested to obtain bead-free PCL-PEI nanofiber surfaces. In literature, Kim et al. used chloroform (Chl) and *N,N*-dimethylformamide (DMF) solvent mixture (Chl:DMF, 3:1, v:v) for the production of PCL-PEI nanofibers to observe NIH 3T3 fibroblast cell line attachment and proliferation of the cells on the nanofiber surface [25]. Jing et al. also used Chl:DMF solvent mixture in the ratio of 3:2 (Chl:DMF, v:v) to construct the scaffold for human umbilical vein endothelial cells (HUVECs) viability evaluation [24].

As shown in Table 1, bead-free and uniform nanofibers were only formed by using PCL-PEI in 2:1 (v/v) ratio. To prove these results, SEM images were taken and contact angle measurements were also performed to evaluate the surface characteristics of these PCL-PEI nanofibers (Fig. 1). Contact angle results were obtained by measuring three different parts of PCL-PEI surfaces for each sample. Contact angles of PCL-PEI (1:1, v/v), PCL-PEI (1:2, v/v) and PCL-PEI (2:1, v/v) surfaces were measured as 53.59° ± 3.17, 49.7° ± 1.44, 44.88° ± 4.04, respectively. Although PCL has highly hydrophobic characteristic, all surfaces showed low contact angle results thanks to the blending of PEI in a mixture.

G. oxydans is a gram negative bacteria, compulsory aerobe which belongs to the family of Acetobacteraceae. Since *G. oxydans* can oxidize many range of substrates regioselectively, it comprises an important portion of industrial and biotechnological applications among other

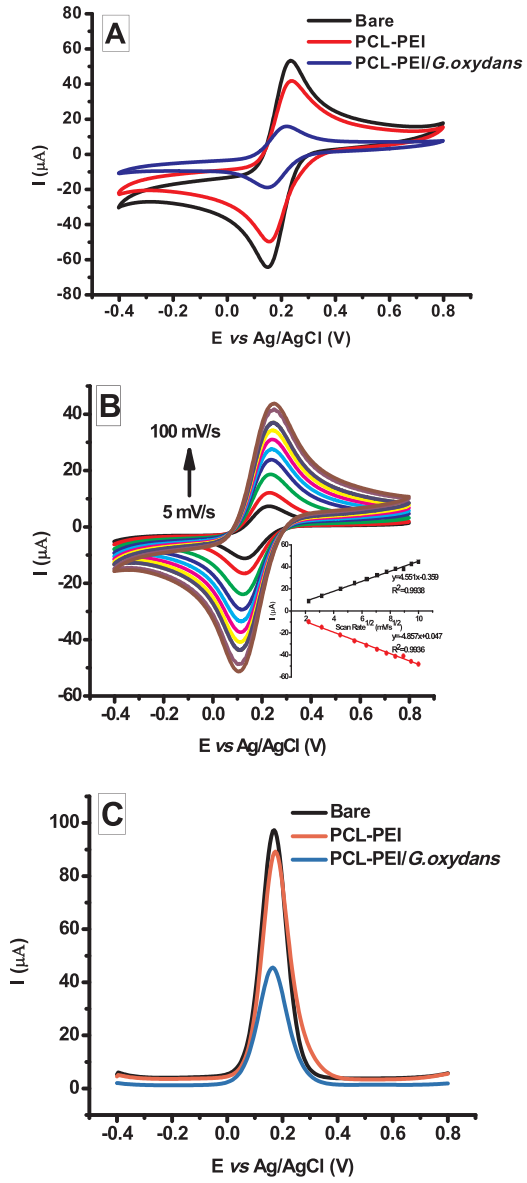

Fig. 3. A) Cyclic voltammograms of bare, PCL-PEI, and PCL-PEI/*G. oxydans* coated GC electrodes (in 5.0 mM $[Fe(CN)6]^{3-/4-}$ and 0.1 M KCl in Na-Acetate buffer pH 5.0, 50 mM at a scan rate of 50 mV/s); B) Cyclic voltammograms of PCL-PEI/*G. oxydans* immobilized on GC electrode at ever-increasing scan rates (The inset graph denotes the correlation between the current and square root of the scan rates of 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mV/s); C) Differential Pulse Voltammograms of bare, PCL-PEI, and PCL-PEI/*G. oxydans* modified GC electrodes (in 5.0 mM $[Fe(CN)6]^{3-/4-}$ and 0.1 M KCl in Na-Acetate buffer pH 5.0, 50 mM at a scan rate of 25 mV/s).

genus. Their morphology can change from ellipsoidal- to rod-shaped structure [32,33]. It can be seen as a single cell, in pairs or in short chains. After the optimization process of electrospun nanofiber preparation, the covalent immobilization procedure of whole cells on the PCL-PEI surfaces was carried out. The success of the immobilization was also proven by using SEM. As shown in Fig. 2, rod-shaped *G. oxydans* can be clearly seen on the PCL-PEI nanofiber after the immobilization process. Furthermore, there was no morphological changes on the bacterium and the PCL-PEI nanofiber surface after covalent immobilization of cells.

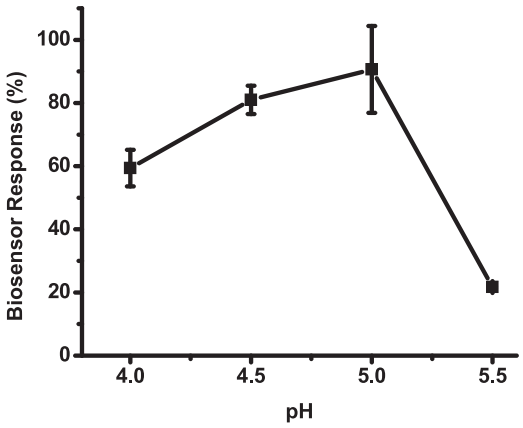

Fig. 4. pH effects on the response of PCL-PEI/*G. oxydans* biosensor (in 50 mM Na-Acetate buffer at ambient conditions, -0.7 V; error bars show S.D. of three measurements).

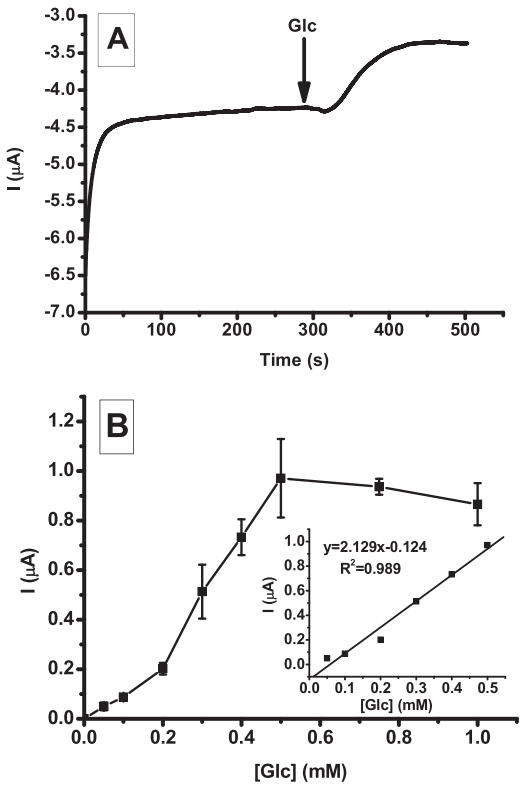

Fig. 5. A) The current change on the PCL-PEI/*G. oxydans* whole-cell biosensor after addition of 0.4 mM glucose (in 50 mM Na-Acetate buffer, pH 5.0, -0.7 V); B) Effect of various glucose concentration on the biosensor response of PCL-PEI/*G. oxydans*. Inset: Linear plot of PCL-PEI/*G. oxydans* whole cell biosensor (in 50 mM Na-Acetate buffer, pH 5.0, -0.7 V). Error bars show S.D. of three measurements.

3.2. Electrochemical characterization of PCL-PEI/G. oxydans covered GC surfaces

To prove the coating of glassy carbon surfaces with PCL-PEI and PCL-PEI/*G. oxydans*, CV and DPV measurements were carried out in the presence of hexacyanoferrate (III) as a redox probe. CV trial was performed with the bare, PCL-PEI and PCL-PEI/*G. oxydans* covered GC electrodes, without stirring, at 50 mV/s vs. Ag/AgCl electrode. Fig.3A depicted the decrease on the current response after each step of modification. Redox peak potential separations were considered for bare, PCL-PEI and PCL-PEI/*G. oxydans* modified surfaces as 86, 82, 70 mV,

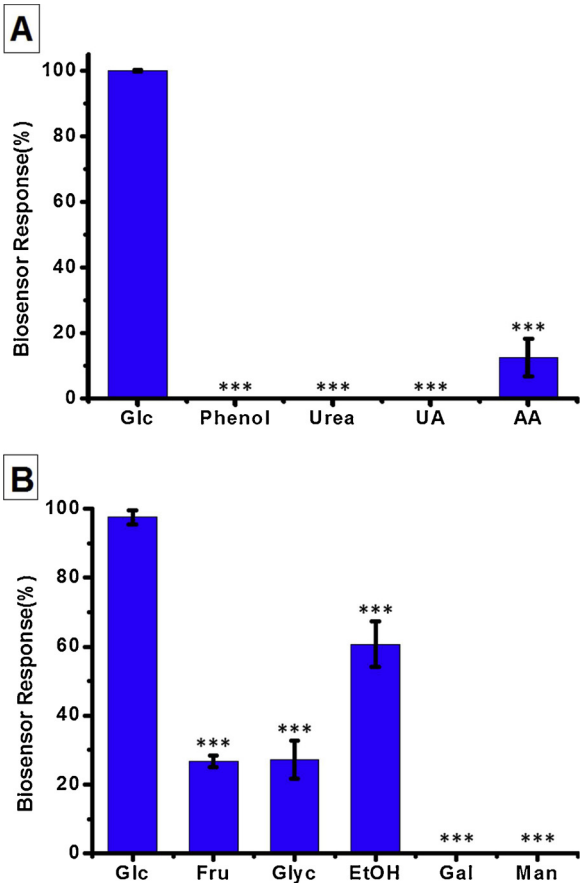

Fig. 6. (A) Interfering effects of some compounds to the glucose signal of PCL-PEI/*G. oxydans* biosensor (1.0 mM phenol, ascorbic acid (AA), uric acid (UA), and urea) in Na-Acetate buffer pH 5.0, -0.7 V); (B) Response percentage of PCL-PEI/*G. oxydans* biosensor to various monosaccharides and alcohols (1.0 mM glucose, fructose, ethanol, glycerol, galactose, and mannose in Na-Acetate buffer pH 5.0, -0.7 V). The asterisk indicates statistical significant when each biosensor response was compared to Glc signal of PCL-PEI/*G. oxydans* biosensor ($p < 0.001$).

Table 2
Analysis of glucose in synthetic biological fluids.

Sample	Added glucose (mM)	Found glucose (mM)	Recovery %
Artificial saliva	2.0	2.02 ± 0.10	99 %
Artificial tears	5.0	5.23 ± 0.63	96 %
Artificial urine	10.0	10.52 ± 0.05	95 %

respectively. The decrease on the peak currents confirmed the successful electrospinning of PCL-PEI and the immobilization of bacteria on the GC electrodes. To authenticate the correlation between the scan rate and the current of PCL-PEI/*G. oxydans* modified GC surfaces, a fresh sensor was prepared and various scan rates were applied to the electrodes. As shown in Fig. 3B, the increased scan rate caused to the increased peak current. The obtained graph between square of scan rate and peak current is depicted in the inset of Fig. 3B. It can be said that diffusion-controlled transfer has been obtained for PCL-PEI/*G. oxydans* modified GC electrodes. After that, DPV measurements were performed with the bare, PCL-PEI and PCL-PEI/*G. oxydans* coated GC surfaces without stirring, between -0.4 and + 0.8 V with scan rate at 50 mV/s vs. Ag/AgCl electrode. Fig.3C indicates the decrease on peak currents after each step of modification. As shown in Fig. 3A and C, there is a significant correlation between current responses before and after coating with PCL-PEI and PCL-PEI/*G. oxydans*. Therefore, it can be said that the successful alteration of GC surfaces with nanofibers and whole-cells has been completed.

3.3. Application of PCL-PEI/G. oxydans on glassy carbon electrode surfaces as a whole-cell sensor

Electrospun nanofibers are mostly used for the preparation of enzyme sensors because of their unique properties. When looking at literature prepare almost all enzyme sensors, enzymes were added into the polymer solution. In our previous study, poly(vinyl)alcohol (PVA) and polyamidoamine (PAMAM) dendrimer intercalated montmorillonite (Mt) clay based (PVA/PAMAM-Mt) electrospun nanofibers were prepared and used as an immobilization material for covalent conjugation of pyranose oxidase enzyme [34]. Binding of biological molecules via covalent bonds to surfaces provides biosensing systems with highest operational stability and repeatability because the enzymes are prevented from breaking off from the surface. The new biosensor system has been prepared taking into consideration this basic principle. PCL-PEI electrospun nanofibers were formed on the surface of GC electrode and *G. oxydans* were immobilized onto them via covalent bonds. First of all, effect of pH on the biosensor response of PCL-PEI/*G. oxydans* whole-cell biosensor system. PCL-PEI/*G. oxydans* was incubated for different buffer systems (pH 4.0–5.5; 50 mM sodium acetate buffers) and the sensor response was determined by injection glucose. As displayed in Fig. 4, working buffer for PCL-PEI/*G. oxydans* was determined as pH 5.0; 50 mM acetate buffer. According to previous *G. oxydans* whole-cell sensors, optimum pH was 7.0 [35], 6.5 [36] and 6.0 [37] as a buffer. The reason for this difference in the pH of the working buffers is materials that have various functional groups. Because the functional group changes the charge of microenvironment on the electrode surfaces [38]. The presence of amine groups on the skeleton of the immobilization matrix caused to shift to acidic sites of the apparent pH. Because positive charge of the PEI interacts with OH^- ions in microenvironments of buffers and the H^+ ions in buffer shifts the pH to acid sites.

As shown in Fig. 5A, the current increased progressively after the glucose was injected into the working buffer. Triplicate measurements were taken for each different glucose concentration in a refreshed buffer. Calibration graph of PCL-PEI/*G. oxydans* was created taking into account the difference in current between the absence and presence of glucose (Fig. 5B). The linear range for glucose is 0.05-0.5 mM with LOD of 48 μM. For the practical application of the proposed PCL-PEI/*G. oxydans*, repeatability of whole cell biosensors is crucial. The repeatability of PCL-PEI/*G. oxydans* was tested in 0.4 mM glucose for 5 chronoamperometric measurements. Standard deviations (S.D.) and coefficient of variation (C.V.) of these measurements were calculated as 0.019 mM and 2.831%, respectively. The immobilization of bacterial cells can be carried out by entrapment, adsorption or covalent binding. Glutaraldehyde is useful cross-linker to immobilize biological molecules on the electrode surface via covalent bonds. Incubation with higher than 5.0% concentration of glutaraldehyde for twenty minutes, it is toxic to bacteria [39]. But using lower concentrations did not affect the viability of cells. In our design, 1.0% glutaraldehyde was used to immobilize *G. oxydans* on the PCL-PEI electrospun nanofibers. Operational stability was tested using PCL-PEI/*G. oxydans* for the analysis of glucose. After 50 measurements during 6 h, no decrease on the biosensor response was obtained. The comparison of some analytical features of *G. oxydans* whole-cell biosensors, which are fabricated using immobilization matrix and electrodes, was summarized in Table S1.

3.4. Selectivity of PCL-PEI/G. oxydans and interferences

As the proof-of-concept, the influence on response current of various potential interferes in samples such as phenol, urea, uric acid and ascorbic acid (AA) was investigated. Except AA, all other compounds did not affect the sensor response of PCL-PEI/*G. oxydans*. The whole-cell

biosensor showed the slight increase on the current after addition of AA (Fig. 6A). To test the selectivity of PCL-PEI/*G. oxydans* against to different sugars and alcohols including fructose, ethanol, glycerol, galactose and mannose, they were injected into working buffer as a substrate instead of glucose. The current change was followed in the absence and the presence of these compounds and the obtained biosensor response was plotted (Fig. 6B). Sensor response was obtained against ethanol as an expected situation since *G. oxydans* has various dehydrogenase enzymes on their cell walls (alcohol dehydrogenase, glycerol dehydrogenase and glucose dehydrogenase etc.) [40]. The results directed that PCL-PEI/*G. oxydans* revealed good selectivity for glucose detection.

3.5. Sample application

To detect glucose in biological fluids using PCL-PEI/*G. oxydans,* artificial urine, tears and saliva solutions were prepared as mentioned before in experimental section [26,27]. After addition of known amount of glucose into samples, they were used as substrate and injected into the working buffer of PCL-PEI/*G. oxydans.* Glucose concentration was 2.0 mM, 5.0 mM and 10 mM in artificial saliva, artificial tears and artificial urine, respectively [41]. The obtained current response values were used to calculate glucose concentration using the calibration curve of glucose in Fig. 5B. The added (known at the beginning) and the calculated glucose concentrations were compared and recovery % of the values were calculated. In Table 2, the results for quantitative analysis of glucose in artificial samples were presented. It can be said that PCL-PEI/*G. oxydans* could be effectively implemented the detection of glucose in samples without any inferences in complex sample matrices.

4. Conclusion

In this study we showed successful integration of microbial cells on the PCL-PEI electrospun nanofibers to construct Gluconosensors. Electrospun nanofibers have attracted considerable attention recentlysince the nanofiber surface provides suitable interfaces between biological component and transducer of biosensors. High surface area to volume ratio, flexibility to surface modification, porous and interconnected structure makes electrospun nanofibers valuable candidates as an immobilization matrix. By taking these advantages of electropsun nanofibers, *G. oxydans* was immobilized by covalent bonds onto the electrospun nanofibers. To obtain a functional group for covalent attachment of bacterial cells, amine groups of PEI were used. Binding on the surfaces of electrospun nanofibers facilitates the oxygen and glucose transfer from buffer to cells. As a result of this binding strategy, sensor response time shortened and LOD for glucose was decreased. As an alternative for *G. oxydans*, various types of bacterial cells can be immobilized on the surface of electrospun nanofibers for bioremediation of heavy metals, dyes, endocrine-disturbing chemicals etc. Also, microbial fuel cells can be constructed and electrospun nanofibers can be used as an ion exchange membrane.

Acknowledgements

Authors acknowledge The Scientific and Technological Research Council of Turkey (TUBITAK; Project 215Z194) and Aliye Uster Foundation for funding this research. M. Gordegir, S. Oz and M. Buhur thank to TUBITAK 2209 A-Program for the University Students at Undergraduate Level. Dr. D. O Demirkol acknowledges the support from the Turkish Academy of Sciences The Young Scientists Award Programme (TUBA-GEBIP2015).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.enzmictec.2019.03.002.

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