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The operation of enzymatic fuel cell fabricated with rationally designed poly(caprolactone-*g*-ethylene glycol) copolymers

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ABSTRACT

This study describes construction of an enzymatic fuel cell comprised of poly(caprolactone-*g*-ethylene glycol) coated novel glucose oxidase anode and laccase cathode. Rationally designed poly(caprolactone-*g*-ethylene glycol) containing various poly(ethylene glycol) percentages ranging between 2.67 and 15.04% were synthesized chemically and tested separately for operation of the fuel cell system to achieve the best energy generation. The maximum power density was found to be 80.55 μW cm^{-2} at 0.91 V (vs. Ag/AgCl) in pH 5, 100 mM citrate buffer (20 °C) by the addition of 30 mM of glucose from the electrodes coated with 11.34% poly(ethylene glycol) containing polymer with a quantity of 600 μg. High poly(ethylene glycol) percentages with more numbers of long poly(ethylene glycol) brushes lead to the creation of a complexity in the polymer morphology and steric hindrance effect for electron transport. The graft copolymer was easily used for the fuel cell system owing to its biocompatible and microporous film morphology. The grafted polymer was able to facilitate enzymatic glucose oxidation and oxygen reduction while simultaneously producing high catalytic electrical currents.

1. Introduction

Enzymatic fuel cells are being intensely studied as prospective power sources for the future of implantable devices [1]. They are energy conversion devices that can efficiently convert the chemical energy of diverse biofuels such as glucose, fructose, cellobiose, alcohol or hydrogen into electrical energy by catalyzing complementary electrochemical reactions [2]. Enzymatic fuel cells consist of two electrode set modified by any stable, electrically conducting or nonconducting material [3]. These materials are used for improving the capability of biomolecule immobilization, electron transfer rate, fuel diffusion and energy generation of the systems. In this context, chemically synthesized polymers can be superior candidates in the design of efficient biofuel cells [4]. These polymers offer many advantages such as high enzyme loading, porous structures, good film properties, effective mass transports, fine control of the polymerization process, versatile covalent modification of the polymer backbone with functional molecules [5]. They were fabricated into various forms of particles, beads, hydrogels, films, and fibers to acquire various the surface morphologies and characteristics in previous biofuel cells [6]. For example, TEMPO immobilized poly(ethylenimine) coated glassy carbon electrode was used as an anode which was capable of generating currents from 0.41 mA cm^{-2} in the presence of 250 mM sucrose, 8.20 mA cm^{-2} in the presence of 2 M methanol and 33.4 mA cm^{-2} in the presence of 500 mM formate. This anode was combined with an enzymatic biocathode to construct a hybrid biofuel cell and produced a current density of 0.38 mA cm^{-2} by using 2 M methanol as fuel source [7]. Ferrocene modified linear poly(ethylenimine) film could be utilized as bioanode in enzymatic fuel cell and produced a power density of 29 μW cm^{-2} [8]. (Os(4,4′-dimethyl-2,2′-bipyridine)$_2$(poly(vinylimidazole))$_{10}$Cl)Cl polymer combined with multiwalled carbon nanotube was used for anode side of an enzymatic fuel cell. The system was generated a current density of 4.2 mA cm^{-2} in 50 mM phosphate buffer in the presence of 5 mM glucose as fuel. The authors claimed that this electrode can be used in enzymatic fuel cells applications for in vivo and ex vivo power generation [9]. Ferrocenylpropyl-modified linear poly(ethylenimine) were used with glucose oxidase in the layer-by-layer assembly of enzymatic bioanode on gold. The system was generated 86 μW cm^{-2} at pH 7.0 and 149 μW cm^{-2} at pH 5.0, when poised against an air-breathing platinum cathode in a compartment-less biofuel cell [10]. In another study, an application of poly(2-hydroxyethyl methacrylate) and ethylene glycol methacrylate phosphate copolymer was developed for a biofuel cell which exhibited maximum power density of 0.2 mW cm^{-2} via the fructose oxidation [11]. Polycaprolactone is one of these polymers which has been widely used in drug carrier system due to the great permeability, biodegradability and nontoxicity. However, the potential applications of polycaprolactone are considerably restricted by the high

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hydrophobicity, rather high crystallinity and the inadequate interaction between polycaprolactone and cells. To overcome this drawback, polycaprolactone/poly(ethylene glycol) graft copolymers have been synthesized in different morphologies in the form of microparticles, nanoparticles, hydrogels, and micelles commonly for drug carrier systems [12]. Poly(ethylene glycol) is a neutral polyether that has been widely used in materials science and biotechnology because of its stability, biocompatibility, water solubility, nontoxicity, rapid clearance from the body, and lack of immunogenicity [13]. Its chains are well known to stabilize proteins, preventing their denaturation and promoting long-term bioactivity [14]. Poly(ethylene glycol) is also exploited as a spacer for protein immobilization. The introduction of this flexible spacer is expected to enhance catalytic activities of the enzymes by offering them greater freedom of movement as well as minimizing unfavorable steric hindrance posed by solid supports [6]. When poly(ethylene glycol) is copolymerized with polycaprolactone, it brings flexibility to the final copolymer chain by affecting the polymeric morphology. For instance, it changes the pores and their size in the cross sections, which can be associated with the chain flexibility of the grafted polycaprolactone [15]. In addition, it is known that the amount and molecular weight of the poly(ethylene glycol) segment in the poly(ethylene glycol-*g*-caprolactone) (PCL-*g*-PEG) copolymer have a pronounced effect on the hydrophilicity and crystallinity of polycaprolactone [16]. Even though, poly(ethylene glycol) attached nanocomposites have the feature of electrical conductivity [17] beside these excellent properties, to the best of our knowledge, its copolymers have not been used as supporting materials of biological fuel cell electrodes.

Based on this background, the focus of this paper is the construction of an enzymatic fuel cell prepared with rationally designed poly(ethylene glycol-*g*-caprolactone) copolymer. A series of the rationally designed poly(ethylene glycol-*g*-caprolactone) was synthesized by changing the percentage of poly(ethylene glycol) blocks in the copolymers which were previously reported by our chemistry group [15]. The polymers were tested for the first time as electrode supporting material in this study. The effect of poly(ethylene glycol) percentage on energy generation performance of the fuel cell was investigated and optimum poly(ethylene glycol) percentage was determined for ideal supporting material of the working electrodes for the generation of maximum power output.

2. Material and methods

2.1. Reagents

Glucose oxidase from *Aspergillus niger* (10 KU), laccase from *Trametes versicolor*, glucose monohydrate, poly(ethylene glycol) methyl ether (Mn: 2000), poly(ε-caprolactone) (Mn: 70,000), sodium azide, propargylamine (98%), CuBr (99.9%), propargyl chloride (70 wt% in toluene), 4-dimethylaminopyridine (99%), 2,2′-azobisisobutyronitrile, n-hexane, hydrochloric acid (37%), potassium permanganate, *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) (99%), *N*,*N*′-dicyclohexylcarbodiimide (99%) tetrahydrofuran (≥99.9%), *N*,*N*-dimethylformamide (DMF) (99.8%) and dichloromethane were provided from Sigma-Aldrich. Citric acid monohydrate, tri-sodium citrate dehydrate and poly(ethylene glycol) (Mn: 2000) were purchased from Merck. Stock solutions of enzymes and glucose were daily prepared in 100 mM pH 5 citrate buffers.

2.2. Rational synthesis of PCL-g-PEG copolymers

Azide terminated polycaprolactone (PCL-N_3) and alkyne terminated poly(ethylene glycol) were synthesized in our previous report [15]. Click coupling reactions of azide terminated polycaprolactone and alkyne terminated poly(ethylene glycol) were carried out using CuBr/PMDETA catalyst. Poly(ethylene glycol) (0.2 g, 0.08 mmol), PMDETA (230 μL, 1.1 mmol), DMF (7 mL) and PCL-N_3 (0.5 g, 0.04 mmol) were added into a Schlenk tube. The mixture was degassed by three freeze-evacuate-thaw cycles and backfilled with argon. 0.1 g of CuBr was then added under argon and the Schlenk tube was sealed. The click reaction was carried out at room temperature for 36 h, and then the polymer solution was diluted with chloroform and passed through alumina column to remove copper salt. The polymer solution was concentrated and precipitated in cold diethyl ether, repeatedly two times. The synthetic route for the synthesis of the PCL-*g*-PEG amphiphilic graft copolymer was presented in Fig. 1. PCL-*g*-PEG copolymers containing various poly(ethylene glycol) ratio were coded as given in Table 1.

2.3. Electrochemical experiments

CHI 1040B model electrochemical analyzer was used for electrochemical analyses. Rectangular platinum plates (1 cm × 2 cm) which have uniform size and shape were used for both anode and cathode of the enzymatic biofuel cell. The enzymatic biofuel cell system was also comprised of a platinum wire counter electrode, Ag/AgCl (3 M NaCl) reference electrode and a conventional electrochemical cell (obtained from CH Instruments firm). The system was operated in 20 mL of 100 mM, pH 5 aerated citrate buffer under continuous stirring at 100 rpm by applying proper potential to anode and cathode, respectively. After reaching to a steady-state background current, predetermined concentration of glucose was added to the electrochemical cell to produce electrical current from the enzymatic biofuel cell system. Cyclic voltammogram experiments were conducted in the same buffer at a potential scan ranging between −0.6 and +0.6 V vs. Ag/AgCl with a scan rate of 100 mV s^{-1}.

2.4. Fabrication of anode and cathode working electrodes

Surface of the platinum plates were polished with slurries of fine gamma alumina powders (1, 0.3 and 0.05 μm size) on a polishing micro-cloth pad then, rinsed with double-distilled water. 60 μL of PCL-*g*-PEG4 polymer solution (10 mg mL^{-1}) was directly spread onto the surface of the platinum plates. The electrodes were allowed to dry for solvent evaporation at room temperature then, washed with double-distilled water. 40 μL of glucose oxidase (10 mg mL^{-1}) and 40 μL of laccase (10 mg mL^{-1}) were dropped onto the PCL-*g*-PEG4 film coated anode and cathode, respectively and waited for enzyme adsorption at room temperature for 2 h. The anode and cathode were washed in 2 mL of 100 mM, pH 5 citrate buffers to remove the unbound enzyme from the electrode surfaces. These flushing waters were stored for protein analysis to determine the amount of immobilized enzymes.

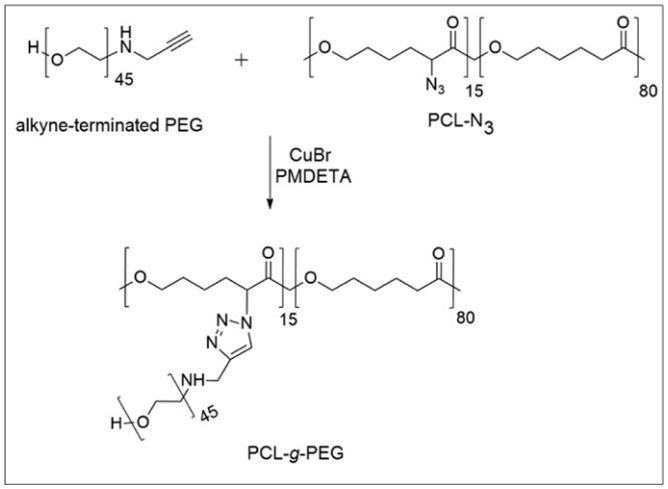

Fig. 1. The synthesis reaction mechanism of PCL-*g*-PEG copolymer.

Table 1
Power generation of the enzymatic fuel cell designed with different poly(ethylene glycol) ratio (%) by the addition of 30 mM glucose.

Polymer	Poly(ethylene glycol) ratio (%)	Power density (μW cm^{-2})
PCL	0	15.24
PCL-*g*-PEG1	2.67	16.20
PCL-*g*-PEG2	4.72	23.71
PCL-*g*-PEG3	7	36.40
PCL-*g*-PEG4	11.34	80.55
PCL-*g*-PEG5	15.04	50.05

3. Results and discussion

3.1. Characterization of working electrode surfaces

The rationally designed PCL-*g*-PEG copolymers were characterized by FT-IR, gel-permeation chromatography, scanning electron microscope, surface tension, contact angle and water uptake measurements, differential scanning calorimeter and thermogravimetric analyses in our recently published report [15]. The structural characteristics of the graft copolymers were evaluated by using ^{1}H NMR spectrometry. Fig. 2 showed ^{1}H NMR spectrum of the PCL-*g*-PEG4 graft copolymer. The characteristic signals of each segment of the copolymer were observed in the spectrum. Chemical shifts in polycaprolactone units can be assigned to the signal of the independent methylene protons at 1.2–1.6 ppm, carboxyl group adjacent methylene protons at 2.2 ppm and oxygen atoms in acyloxy group adjacent to methylene protons at 4.0 ppm. Poly(ethylene glycol) and triazole proton signals were observed at 3.6 ppm and 7.9, respectively. ^{1}H NMR spectrums of the PCL-*g*-PEG copolymers containing various poly(ethylene glycol) percentages (number of units) were presented in Supporting information file. Poly(ethylene glycol) percentages attached to the PCL-*g*-PEG copolymers were calculated from integration values by ^{1}H NMR spectras. It was observed that the number of ester bond formed in the alkyne terminated poly(ethylene glycol) affected the poly(ethylene glycol) percentage attached to the polycaprolactone chain.

Scanning electron microscope images of only PCL-*g*-PEG4 coated, glucose oxidase immobilized and laccase immobilized PCL-*g*-PEG4 coated platinum surfaces were taken with Quanta FEG 450 model scanning electron microscope, and presented in Fig. 3A, B and C, respectively. All scanning electron microscope images showed that the polymeric layer has a microporous structure. Especially, poly(ethylene glycol) brushes of the polycaprolactone based copolymer were clearly seen in the enzymeless polymeric film coated electrode surface. Different surface morphologies were observed for enzyme immobilized polymeric film surfaces. In our previous study, the internal morphologies of the PCL-*g*-PEG with varied poly(ethylene glycol) ratio were presented with scanning electron microscope images. The results showed that there was a distinct difference in pore size in each image, and the pore sizes of the copolymers ranged between 350 and 400 nm with varying percentages of poly(ethylene glycol) [15].

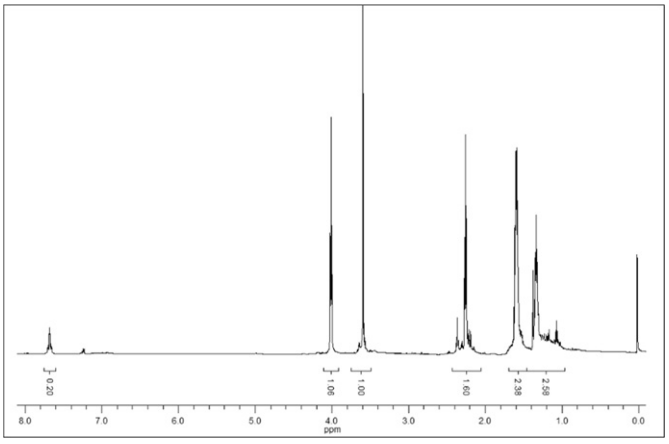

Fig. 2. ^{1}H NMR spectrum of the PCL-*g*-PEG4 graft copolymer.

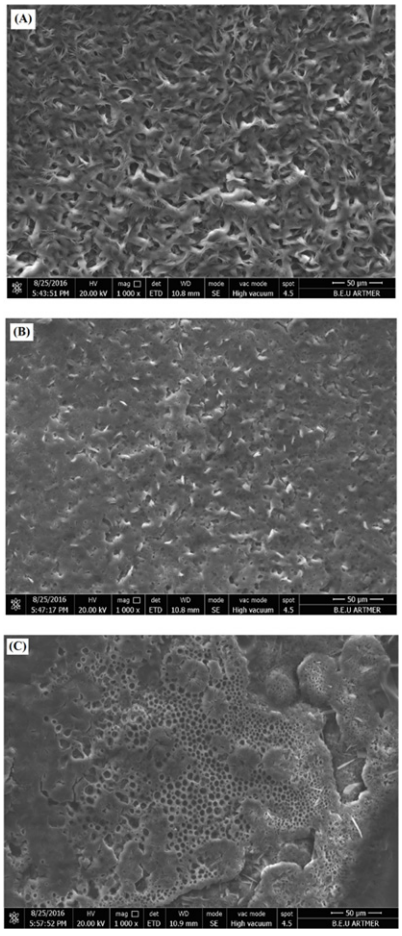

Fig. 3. Scanning electron microscope image of PCL-*g*-PEG4 (A), glucose oxidase immobilized PCL-*g*-PEG4 (B), laccase immobilized PCL-*g*-PEG4 (C) coated electrode surface taken from with a magnification of 50 μm.

In an enzymatic fuel cell which has a direct electron transfer mechanism comprised of glucose oxidase anode and laccase cathode, anodic enzyme is responsible for transferring electrons from the fuel to the anode by catalytically oxidizing fuel under an applied anodic potential. Electrons flow through to cathode where molecular oxygen is catalytically reduced by laccase. An electrical current is generated as a result of this electron flow. The magnitude of the electrical current mainly depends on the fuel concentration, electron transfer ability of polymeric film layer, enzymatic activity and the cell potential. Bioelectrochemical reactions realized on the electrode surfaces were presented below:

$$\text{Anode reaction}: C_6H_{12}O_6 \rightarrow C_6H_{10}O_6 + 2H^+ + 2e^- \quad (1)$$

$$\text{Cathode reaction}: \frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O \quad (2)$$

The electrochemical behavior of the glucose oxidase immobilized PCL-*g*-PEG4 coated anode was investigated. The cyclic voltammogram experiment in presence of 30 and 50 mM of glucose was conducted at a potential scan between −0.6 and +0.6 V vs. Ag/AgCl in 100 mM, pH 5 citrate with a scan rate of 100 mV s^{-1} (Fig. 4). It was clearly seen from Fig. 4 that the anode exhibited a high activity for glucose oxidation. The current difference between 30 mM and 50 mM glucose oxidation

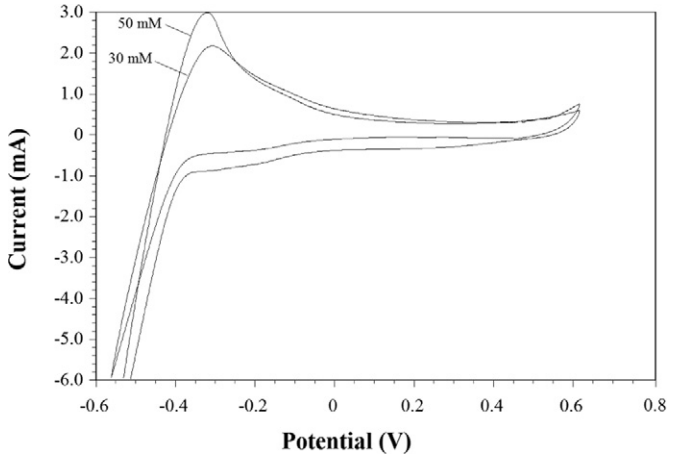

Fig. 4. Cyclic voltammogram of the PCL-*g*-PEG4/glucose oxidase anode in 20 mL of 100 mM citrate buffer (pH 5) in presence of 30 mM and 50 mM glucose at a potential scan ranging between −0.6 and +0.6 V (vs. Ag/AgCl) with the scan rate of 100 mV s^{-1}.

was at mA level. Glucose oxidation effectively occurred at −0.32 V. This result is consistent with the theoretical potential value of glucose oxidation by glucose oxidase [18]. It was understood that the polymeric film layer did not create a resistance for effective oxidation of glucose on the electrode surface. Surface concentrations of glucose on the anode were determined by using the expression defined by Laviron [19]:

$$i = -nFA\frac{d\Gamma}{dt} \quad (3)$$

Γ is the surface concentration of glucose (mol cm^{-2}), $\frac{d\Gamma}{dt}$ is glucose transfer rate onto the electrode surface (mol cm^{-2} s^{-1}), A is the electrode area (cm^2), n is the number of electron transferred to the anode via fuel, F is Faraday constant (96.485C) and i is the oxidation current expressed in Ampere. Assuming that, 1 mol of glucose was oxidized by releasing 2 mol of electrons (Eq. (1)), platinium anode surface was 2 cm^2 and glucose oxidation current was 2 mA and 3 mA for 30 mM and 50 mM of glucose, respectively. The results showed that the surface concentration of the fuel transferred in 1 s was found to be 1 μg cm^{-2} (for 30 mM glucose addition) and 1.4 μg cm^{-2} (for 50 mM glucose addition). Enzymless anode was tested as control electrode. Results showed that no current generation was observed by the addition of various concentrations of glucose.

3.2. Effect of poly(ethylene glycol) ratio on energy generation

PCL-*g*-PEG copolymers including various poly(ethylene glycol) ratio (2.67%, 4.72%, 7%, 11.34% and 15.04%) were tested in the fuel cell system. The working electrodes were fabricated according to the method given in Section 2.4. Power generation ability of the electrodes coated with pure polycaprolactone was also investigated. The system was operated in 20 mL of 100 mM, pH 5 citrate buffer containing 30 mM of glucose by applying (−0.32 V)/(+0.59 V) (anodic/cathodic) potential. The generated electrical currents at various poly(ethylene glycol) ratio were presented in Fig. 5A, and the obtained power density (defined as the product of the cell potential and the generated current density) of the each fuel cell was shown in Table 1. The fuel cell electrodes prepared with pure polycaprolactone generated the minimum power in comparison to the poly(ethylene glycol) containing electrodes. One of the reason was the grafting poly(ethylene glycol) units increased the hydrophilicity of the polymeric layer [15], thus contributing to increase the diffusivity of the analyte. The generated current and power density increased with increasing poly(ethylene glycol) ratio up to 11.34%. In our previous study, the characterization experiments showed that

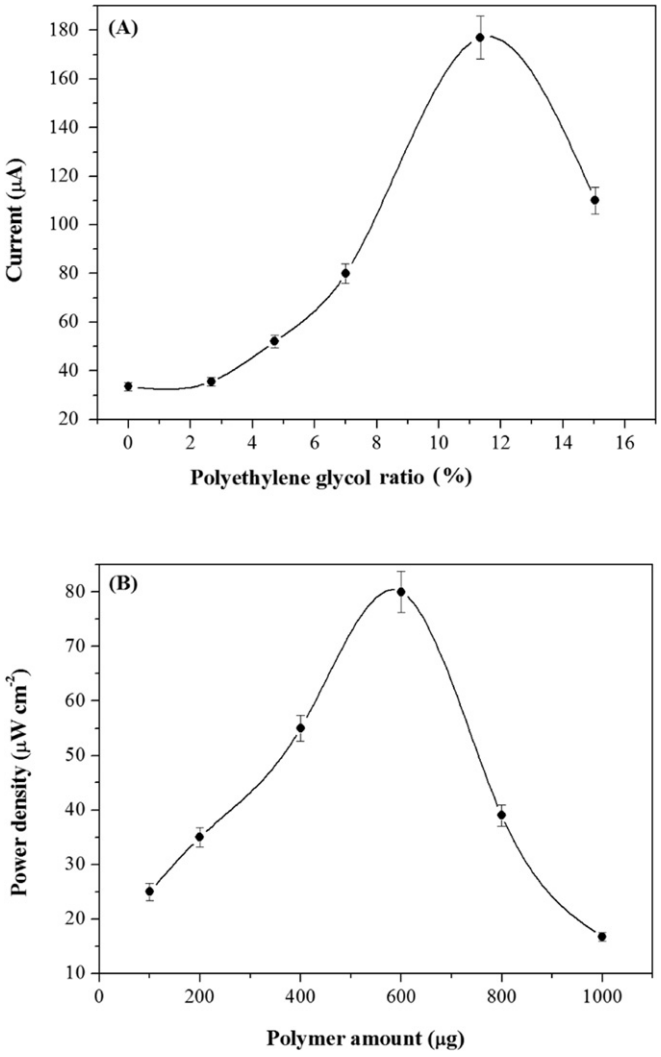

Fig. 5. Current generations of the working electrodes coated with various PCL-*g*-PEG polymers containing varied percentage of poly(ethylene glycol) (%) (A), power densities of enzymatic fuel cell series fabricated with different PCL-*g*-PEG4 polymer amounts (B) in presence of 30 mM glucose (cell voltage: 0.91 V vs. Ag/AgCl).

poly(ethylene glycol) content in the polycaprolactone film affected the polymer morphology. In PCL-*g*-PEG polymers, the pores and their size in the cross sections increased with the increasing amount of poly(ethylene glycol) in the polymer chain [15]. In this case, the fuel could be diffused efficiently into the polymeric layer, and hence, enzymatic fuel oxidation rate accelerated. The side poly(ethylene glycol) chains created polymer brushes in PCL-*g*-PEG and the number of these brushes was proportional with poly(ethylene glycol) ratio in the copolymer chain. These brushes provided larger surface area for better biocatalytic activity and enhanced electron transfer process on the working electrode surface. In addition, poly(ethylene glycol) chains are well known to stabilize proteins, preventing their denaturation and promoting long-term bioactivity [14]. However, the fuel cell comprised of working electrodes fabricated with poly(ethylene glycol) ratio of 15.04% generated a lower power density in comparison to poly(-ethylene glycol) ratio of 11.34%. This can be attributed to the creation of a complexity in the polymer morphology and a steric hindrance effect for electron transport on the electrode surface due to the more numbers of long poly(ethylene glycol) brushes. PCL-*g*-PEG4 polymer synthesized with a poly(ethylene glycol) ratio of 11.34% is the most suitable layer to generate the highest electrical energy for the enzymatic fuel cell designed in this work.

Table 2
Power output of the glucose oxidase/laccase based biofuel cells in previous studies.

Polymeric film	Power density (μW $cm^{-2)}$	Reference
Polyaniline nanofiber	37.4	[22]
Carbon nanotube/hydroxyapatite nanocomposite	15.8	[23]
Lyotropic liquid crystalline cubic phase	7	[24]
Polypyrrole	27	[25]
Os(4,4-diamino-2,2′ bipyridine)$_2$(poly{*N*-vinylimidazole})-(poly{*N*-vinylimidazole})$_9$Cl	40	[3]
Nanographene platelets	57.8	[26]
Ferrocene based matrix	13	[27]
Osmium based matrix	3.5	[28]
Carbon nanotube/ferrocenecarboxaldehyde silk film	50.7	[29]

3.3. Effect of polymer quantity on energy generation, and investigation of power generation capacity of the system

Polymeric film layer enables enzyme immobilization and also provides a biocompatible microenvironment for a controllable access of fuel and molecular oxygen on working electrode surface. However, coating of the working electrode surfaces with especially nonconducting and chemically synthesized polymers requires careful attention to avoid poorly conducting surfaces which provide inefficient electron transfer and diffusional barriers [20]. Therefore, it is important to investigate the polymer quantity to be involved on the electrode surface. Enzymatic fuel cells were designed in a series by coating platinum electrodes with various amounts of PCL-*g*-PEG4 polymer (100–200–400–600–800 and 1000 μg). 40 μL of glucose oxidase (10 mg mL^{-1}) and 40 μL of laccase (10 mg mL^{-1}) were dropped onto the anode and cathode, respectively for each system. The fuel cell electrodes were tested by adding of 30 mM glucose into the cell compartment filled with 20 mL, 100 mM, pH 5 aerated citrate buffer at an applied potential of −0.32 V for anode and +0.59 V for cathode. Power densities generated from the fuel cells were presented in Fig. 5B. Power generation increased with increasing polymer amounts up to 600 μg, and then decreased at polymer amounts higher than 600 μg. The additional polymer quantities likely created steric constraints with regard to glucose diffusion; in addition, by considering that the PCL-*g*-PEG was basically a nonconductive polymer owing to polycaprolactone units, probably a hindrance effect was created for electron transport occurring between enzyme and electrode. Thus the power output of the biofuel cell was reduced further.

Maximum power density was calculated to be 80.55 μW cm^{-2} from the fuel cell electrodes coated with 600 μg of PCL-*g*-PEG4 polymer. The immobilized glucose oxidase and laccase quantity calculated by using Bradford Protein Assay [21] was found to be 76 μg and 30 μg, respectively. The obtained power density was higher than previously published similar studies, even though various redox mediators were used to promote electron transfer rate on the electrode surfaces in those (Table 2). It is known that poly(ethylene glycol) modified surfaces render surface protein resistant and enhance surface biocompatibility [30]. For example, a carbon paste electrode incorporating poly(ethylene glycol) modified glucose oxidase exhibited higher response than unmodified electrode for glucose oxidation [31]. Xiao et al. [32] reported a highly sensitive and selective method to detect dopamine in the presence of ascorbic acid by polymeric composite/poly(ethylene glycol) modified electrode. In another study, poly(ethylene glycol) modified electrode showed good electrocatalytic oxidation of dopamine in comparison to unmodified electrode [30]. Poly(ethylene glycol) contributed to high energy output from the enzymatic fuel cell designed in this work even though polycaprolactone units were nonconductive.

Enzymatic fuel cell electrodes coated with 600 μg of PCL-*g*-PEG4 copolymer were operated in 20 mL of 100 mM, pH 5 aerated citrate buffer at the anode/cathode potential of −0.32/+0.59 V (+0.59 V is the theoretical potential of laccase catalyzed oxygen reduction [33]). The system was waited for reaching to a steady-state current value under the constant stirring. Then, various concentrations of glucose ranging between 10 and 60 mM were added successively into the cell to produce current-time recordings. The relationship between the glucose oxidation current and the glucose concentration at the anode side followed the Michaelis-Menten kinetic mechanism (Fig. 6). Glucose oxidation current increased up to the glucose concentration of 40 mM significantly, and then a little increase was observed at the glucose concentration ranging between 40 mM and 60 mM. The apparent Michaelis-Menten constant (K_m^{app}) could be estimated from Fig. 6. Maximum oxidation current (I_{max}) was observed as 210 μA, and K_m^{app} value was calculated to be 8 mM for immobilized glucose oxidase. Glucose oxidation reaction at the anode side was at zero order for glucose concentrations beyond 40 mM. The oxygen reduction is the desired reaction at the cathode side of biofuel cell systems. It provides typically the most attractive electron acceptor reaction to combine with the fuel oxidation reaction at the anode side. Electrons flowing through to the cathode designate the electrical generation capacity of a fuel cell. Therefore, the cathode performance is the identifier of the overall system performance. Laccase has an active site which is characterized with its redox center contains Cu (II) ion where oxygen is reduced to water [34]. High-performance biological fuel cells require efficient electron transfer between the enzyme active site and the electrode, as well as the efficient supply of laccase with oxygen [5]. The four-electron reduction of oxygen to water catalyzed by laccase represented the cathodic half-cell reaction of the fuel cell. The reduction current of the PCL-*g*-PEG4/laccase cathode was presented in Fig. 7. It is possible to observe a significant increase of the reduction current at increasing glucose concentration up to 40 mM. Any further increase of the concentration of glucose did not show any

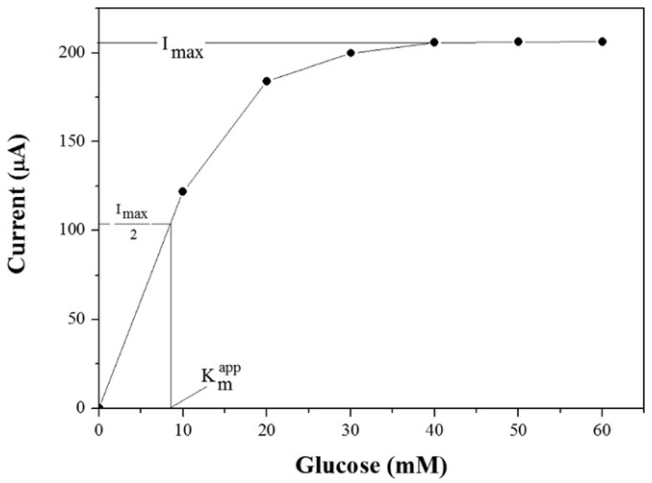

Fig. 6. Effect of glucose concentration on enzymatic glucose oxidation current based upon Michaelis-Menten kinetics. When concentration equals K_m^{app}, I is one-half of the maximum current I_{max}.

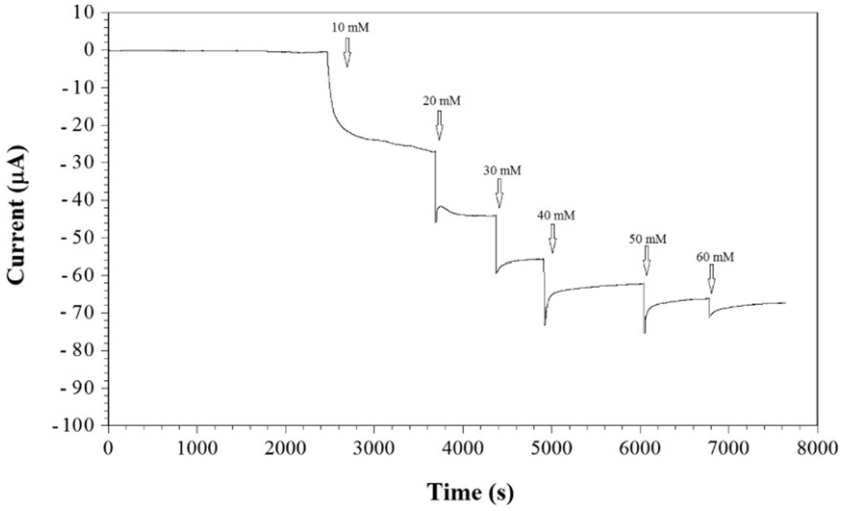

Fig. 7. Oxygen reduction currents of the PCL-*g*-PEG4/laccase cathode obtained from the increasing glucose concentrations ranging between 10 and 60 mM (cell voltage: 0.91 V vs. Ag/AgCl).

significant increase of the oxygen reduction current at the cathode. This behavior was not surprising since all of the accessible immobilized glucose oxidase was converted to enzyme-substrate complex at this glucose concentration level at the anode side (Fig. 6). As a result of this, the flowing number of electrons required for oxygen reduction at the cathode was maximum at this concentration level by considering that the operation medium was comprised of oxygen saturated buffer and the reduction reaction was not oxygen-limited. Even though, the direct electron transfer of laccase is generally difficult due to the complex structure of its redox center and the unfavorable orientations of laccase on cathode surfaces, in this study, the oxygen reduction current was observed to be at the high μA level as a result of the fast and effective electron transport toward to the cathode side.

The operational stability of the anode was performed by the successive addition of 30 mM glucose ($n = 6$) into the working buffer. A reproducible oxidation current with a relative standard deviation (RSD) of 1.1% was observed in 6 successive assays. The electrode was rinsed with working buffer and stored at 4 °C in refrigerator for storage stability test over 20 days period by monitoring the glucose oxidation current generated in presence of 30 mM glucose. The anodic current maintained 97% of its initial value up to 20 days.

4. Conclusion

Rationally designed poly(caprolactone-*g*-ethylene glycol) was synthesized and tested for the first time for enzymatic biofuel cell electrodes which operated with glucose. Maximum energy generation was observed from the enzymatic biofuel cell fabricated with poly(caprolactone-*g*-ethylene glycol) which contained 11.34% of poly(ethylene glycol). The designed enzymatic biofuel cell generated a power density of 80.55 μW cm^{-2} with 30 mM of standard glucose at the cell voltage of 0.91 V. Enzyme-friendly poly(ethylene glycol) chains provided a microporous and hydrophilic structure to the polycaprolactone polymer for facile fuel diffusion and enzyme immobilization in mild conditions on the electrode surface.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2017.03.117.

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