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Research Paper

Implementation of Redox-Enzyme Electrodes in Biosensors and Enzymatic Biofuel Cells

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Abstract

The successful implementation of redox-enzyme electrodes in biosensors and enzymatic bio fuel cells has been the subject of extensive research. This paper presents three new design concepts of a glucose oxidase (GOx) electrode as an anode for the biofuel cell. We compare three enzyme fuel cell (EFC) device based on glucose as fuel and glucose oxidase (GOx) as biocatalyst. Miniature biofuel cells might be an excellent alternative energy supply sources for microelectronic devices. Polarization and power density curves of the three complete EFC devices were acquired, demonstrating the suitability of the immobilization strategy and materials to be used in EFCs. Future work will be regarding the implementation of the enzyme hPG, enzyme fuel cell (EFC) and enzyme FAD/FADH2 electrodes in a micro biofuel cell for health care applications.

Keywords: Glucose oxidase; Biofuel cell; Direct electron transfer; Enzyme immobilization.

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1. Introduction

The functional immobilisation of redox enzymes, such as laccase and glucose oxidase, onto electrode material surfaces is of keen interest for sensors and biofuel cells development. Enzymes are the most common bioreceptor molecules used in biosensors due to their extremely high specificity that leads to minimal risk of false positive responses. The implementation of enzymes in biofuel cells allows for the development of membrane-less and compartment less devices, which not only can be easily miniaturised, but can also be used in situations where it is not feasible to separate the fuel and oxidant [1]. GOx is an oxidoreductase-type enzyme commonly found in many fungus and bacteria [2]. Its substrate (glucose) is readily available, thus offering interesting applicative perspective in terms of cost reduction and sustainability. On the other hand, GOx does not directly transfer electrons to conventional electrodes needing the presence of external redox mediators and their leakage from electrode and instability eventually lead to toxicity issues and poor EFC performance over time [2]. In this respect, glucose oxidase (GOx)may be the best enzyme for the biofuel cells. GOx is stable, highly catalytically active, inexpensive, and glucose as a fuel is wide spread in the biological environment [3] .The successful implementation of redox-enzyme electrodes in biosensors and enzymatic biofuel cells has been the subject of extensive research.[3] The main benefits of enzyme-based biofuel cells are the capability to produce biofuel cells orders of magnitude smaller than equivalently pow-ered microbial cells and allowing operation to

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take place closer to the redox potential of the enzyme itself.[3]To obtain direct electron transfer between the redox center of GOx and electrode surface we have exploited the combination of the excellent properties of carbon nano-tubes in terms of dimension, stability and electrical conductivity [2]. Direct electron transfer (DET) of electrons between the active site of the enzyme and the electrode surface is attrac-tive due to simplicity. It has an excellent potential for miniaturization and high power-output [3]. The process of bio electrocatalysis requires a developed media-tion and enzyme immobilization methodology for continu-ous and efficient electron transfer from the enzyme to the electrode surface [3].

2. Glucose Oxidase Directly Immobilized onto Highly Porous Gold Electrodes 2-1. Immobilisation of GOx

GOx was electrochemically adsorbed onto the prepared hPG disk electrodes by conducting a total of 6 CV scans between 0.42 V and 0.60 V (vs. SCE) at a scan rate, in a PBS solution containing GOx .As a term of comparison of performance, GOx was also immo-bilised by absorption. In this case, the hPG electrodes were incubated with the GOx solution in PBS for 1 hour at room temperature, without conducting any CV scans. In both cases, the GOx-hPG electrodes were then thoroughly rinsed three times with PBS to remove any weakly bonded enzyme, and stored in PBS at 4 ◦ C until used.The amount of GOx immobilised onto the hPG electrodes, was estimated by performing a kinetic assay. of the enzyme solution before and after the immobilisation procedure and assuming no enzyme losses during the process [1].

2.1. Bio fuel Cell Setup

The biofuel cell consisted of a glass vial con-taining a GOx abs-hPG electrode and a LAC-hPG electrode as anode and cathode respectively. The electrode spacing was approximately. The system was fed with PBS solu-tion containing glucose at pH 7.

The potential difference between the two electrodes was measured in open circuit mode until steady state conditions were achieved. Afterwards, the cell potential was measured under a range of different external resistances applied to the system [1].

2.2. Results

The GOx_{ads}-hPG and the LAC-hPG electrodes were subsequently tested as anode and cathode respectively of a glucose/oxygen enzymatic bio fuel cell. The electrodes were immerged in an aerated PBS solution containing 27.8 mM of glucose, and the potential difference of the cell was monitored by means of a potentiostat (Fig1).An open circuit voltage of 0.58 V was observed, comparable with the value previously reported [27]. Subsequently, the cell was polarized by connecting the electrodes to a range of external resistors. (Fig. 1B) shows the cell voltage and the power density as a function ofthe current density. As shown, the peak power density was of6 µW cm−2at 0.2 V vs SCE. This result, although obtained with a very simple design, is comparable with recently reported miniature enzymatic bio fuel cells. Preliminary experiments encourage the implementation of the GOx_{ads}-hPG electrode as the anode of an enzymatic bio fuel cell. When the electrode was coupled with a LAC-hPG electrode as a cathode, a peak power density of 6 µW cm−2at 0.2 V vs SCE was achieved. Future work will be regarding the implementation of the enzyme hPG electrodes in a micro bio fuel cell for health care applications [1].

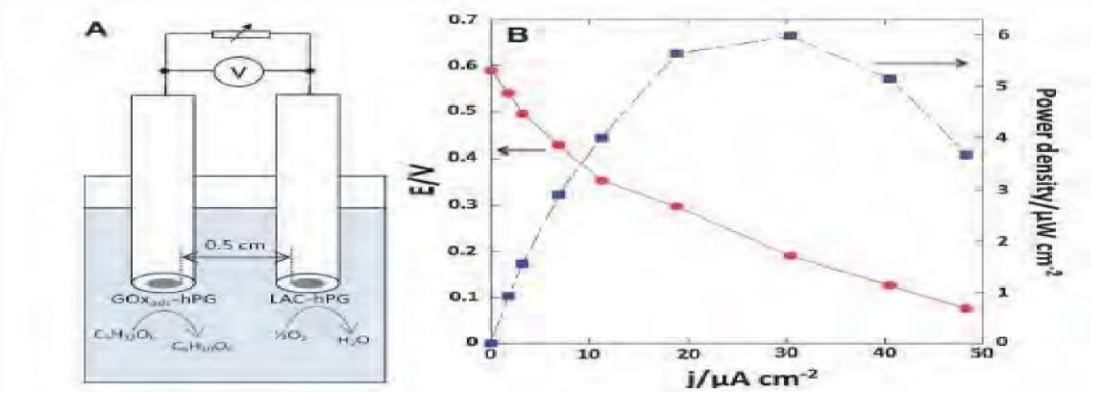

Figure1. Scheme of the enzymatic biofuel cell set up. B: Polarisation and power density curve of the GO_{xads} hPG and LAC-hPG biofuel cell. The power and the current densityrefer to the total superficial surface area of the electrodes used (0.03 cm2).

3. Glucose Oxidase Directly Immobilized onto fuel cell (EFC) by casting the corresponding solutions on carbon cloth 3-1. Immobilisation of GOx

FTIR spectra of both GOx powder and film samples were recorded by using a Perkin-Elmer Spectrum 100 spectro photometer . Film samples were prepared casting N and N-GOx solutions on petri dishes. After solvent evaporation , films were peeled off the support, cut into small pieces and pelleted with KBr. Far-UV CD spectra of both solution and films were recorded with a Jasco spectropolarimeter. A path length quartz cell was used for solution samples. The enzyme concentration used for solution samples and their spectra are showed in molar ellipticity (h). Film samples were prepared by dropping solutions on quartz slides and allowing the solvent to evaporate. The spectra of film samples were normalized by the spectra of free GOx in solution. All spectra were recorded after accumulation of four runs,and smoothed using a fast Fourier transform (FFT) filter to minimize background effects Quantitative prediction of the secondary structure was performed by deconvolution of the CD spectra using CONTINLL, CDSSTR, and SelCon programs [30]. The better results were achieved from CONTINLL program, with a root mean square difference between the experimental and calculated curves lower than 5 % for all deconvolutions. Crystallographic structure of GOx was generated using RASMOL software . SEM images were acquired using FE-SEM, LEO mod. Supra 35, being 15 kV the electron beam energy. Cyclic voltammetry (CV) and chronoamperometry (CA) experiments were performed using a multichannel potentiostatVMP3. CVcurves were recorded in the 0.2–1.0 V range, at a scan rate of 50 mV s1 in the presence of glucose (0–140 mM). CA curves were recorded at the oxidation potential of 0.5 Vin the presence of glucose ranging from 0 to 140 mM. The electrochemical cell, placed in an oven at 30 C, incorporated a conventional three-electrode system, using a saturated calomel reference electrode and a platinum counter electrode. The working electrode consisted in carbon cloth modified with N-GOx or N-GOx-MWNTs, obtained by casting the corresponding solutions on carbon cloth supports. Carbon cloths are either E-Tek ELAT HT 140E-W with a platinum loading of 5 gm-2 carbon cloth (henceforward named as CC-Pt) or carbon cloth plain, Fuel Cell Scientific, Stoneham, MA(henceforward named as CC) [2].

3.1. Bio Fuel Cell Setup (EFC)

The EFC device used for acquiring polarization and power density curves consists in two plexiglass chambers separated by the electrolyte membrane. All solutions were prepared in PBS. The catholyte chamber consisted in an unmodified carbon cloth electrode immersed in potassium ferricyanide solution. Two different anolyte chambers were tested:(a) mediatoradded anolyte and mediator-less anolyte. The first one consisted in a CC electrode modified with either N-GOx-f or N-GOx-MWCTs-f immersed in a solution containing glucose and methylene blue solution as redox mediator. The second one consisted in a CC electrode modified with either N-GOx-f or N-GOx-MWCTs-f immersed in a solution containing glucose solution. The cells were allowed to equilibrate at leastto obtain stable open circuit voltage (OCV). By applying an external resistance (Rext) and gradually reducing the load , a set of voltage (V) data as a function of resistance was obtained. To achieve polarization and power density curves, current values were obtained by Ohm's law.

3.2. Results

Bio anodes (either N-GOx$_f$ or N-GOx-MWCNTs-f) and SPEEK membranes were assembled in an EFC to test electrochemical performance of materials at 30 _C. The resulting polarization and power density curves are shown in Fig.2. OCV values of 0.2 V were recorded for both cells. The maximum power density obtained from N-GOx-f bioanode was 5 lW cm-2 and the current density value at E = 60 mV was 88 lA cm-2, while N-GOxMWCNTs-f bioanode allowed achieving higher performance leading to a 68 % increase in PD. The improved performance of N-GOx- MWCNTs-f bioanode with respect to N-GOx-f can be ascribed to the presence of MWCNTS at the electrode which enhances GOx bioelectrocatalytic efficiency.The enhanced bioelectrocatalytic efficiency of GOx resulted indeed in an improved electrochemical performance of the EFC device. Enzyme fuel cell devices described in Fig. 2 contain a redox mediator in the anodic chamber, such as methylene blue. To explore if MWCNTs at the electrode are able to perform the same role of the redox mediators and thus to substitute it, we prepared an anodic chamber using mediator less either N-GOx-MWCNTs-f or N-GOx-f as bioanodes and SPEEK as electrolyte membrane. While the cell assembled with N-GOx-f does not produce any detectable current, the EFC device based on mediator-less N-GOx MWCNTs bioanode allows achieving a maximum PD of 1.5 lW cm-2 and current density of 23 lA cm-2 at E =60 mV (Fig. 3).

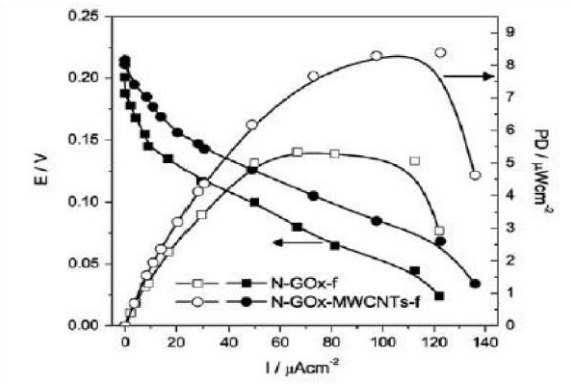

Figure 2. Polarization and power density (PD) curves acquired at 30 C using N -GOx-f and N-GOx-MWCTs-f on CC as bioanodes. Methylene blue was used as redox mediator in bioanode chamber. Fuel 1 M glucose solution

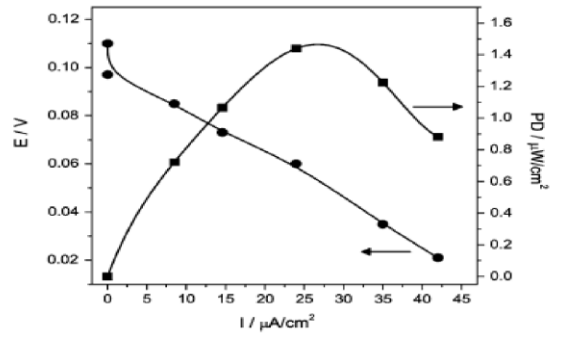

Figure 3. Polarization and power density (PD) curves acquired at30C using SPEEK as electrolyte membrane and mediator less N-GOx-MWCTs-f on CC as bio anode. Fuel 1 M glucose solution.

4. Glucose Oxidase Directly immobilized onto carbon paper modified with MWNTs 4-1. Immobilisation of GOx

Glucose oxidase was immobilized on the Toray carbon paper modified with MWNTs based on the following tech-nique; the carbon paper with incorporated MWNTs were cut into diameter disks. The geometric area of each electrode. The deposition of glucose oxidase molecules (GOx) on the TP/MWNTs surface was carried out by entrapment of the enzyme within polyethyleneimine (PEI) film. The positive charged PEI was used as a binder between the negatively charged GOx enzymes and the TP/ MWNTs electrode surface. GOx was mixed with PEI solution prepared in phosphate buffer. A liquot of GOx PEI solution was cast on both sides of the TP/MWNTs surface and the TP/MWNTs/PEI/GOx disks where then dried at room temperature. Then, Tetrabutylammonium bromide salt treated Nafion solution was cast on TP/MWNTs/PEI/GOx surface and stored at room temper-ature before use. Finally, a TP/MWNTs/PEI/GOx /Nafion disk was inserted into a Teflon tube with a sealing platinum wire connector and this was used as a GOx working electrode. The tetrabutylammonium bromide salt-treated Nafion was used as an ion conductor with a less acidic environment and as an electrochemistry promot-ing polymeric binder. The tetrabutylammonium bromide salt-treated Nafion film is more biocompatible with immobilized GOx and it provides strong and stable adhesion of multi-walled carbon nanotubes and enzyme molecules on the TP surface. The surface morphology of the TP/MWNTs/PEI/GOx/ Nafion disks was investigated by a Hitachi (S-5200) scan-ning electron microscope equipped with a PCT spectrome-ter. The microscope was operated at 10 kV for imaging.The surface concentration of GOx was calculated from the following equation:C ¼ Q= nFA ;where C is the surface concentration of GOx, Q is the charge obtained from integration of the anodic peak, n is the number of electrons per GOx molecule,F is Faraday constant, and A is the electrode surface area. The electro-chemical accessible surface area was calculated using the capacitance of the electrode obtained from cyclic voltam-metry and the specific capacitance for carbonaceous mate-rial to be 20lF/cm2. The coulometric assay has shown that the surface concentration of the glucose oxidase is 0.7×10^{-12} mol/cm2 [3].

4.1 Bio fuel Cell Setup

Electrochemical measurements were performed with Potentiostat/Galvanostat (PAR Model 263A) in a conventional three electrode cell, which included TP/MWNTs/PEI/GOx/Nafionworking, platinum net counter and Ag/AgCl reference electrodes. At the start of the cyclic voltammetry experiments nitrogen was bubbled through the solution. The cyclic voltammetry was used to demonstrate direct electron transfer. Mediated electron transfer was performed to validate overall cata-lytic activity of the immobilized enzyme. Galvanostatic measurements were performed starting at open circuit under constant stirring. To imitate load, different resis-tances were switched into the external circuit. The working solution phosphate buffer containing. All electrochemical experiments. Data of cyclic voltammogram were used to calculate the electron transfer rate constant using the method of Laviron [3].

4.2. Results

The application of the TP/MWNTs/PEI/GOx/Nafion_ modified electrode as an anode for the biofuel cell has been demonstrated during GOx electrode testing in galvanostatic regime (Fig. 6). Analysis has shown that the catalytic electrooxidation current of glucose appears at _380 mV with a current density of 0.01 mA/cm2 and reaches 0.4 mA/cm2 at _120

mV vs. Ag/AgCl. Current density was calculated versus geometric electrode area, giving 0.1 cm2.
The open circuit potential (_400 mV) of the glucose oxidase modified electrode is close to the redox potential of the FAD/FADH2 cofactor in the enzyme itself. Thus, new GOx electrodes based on direct mediatorless electron transfer between the active site of the enzyme and multiwalled carbon nanotubes offer promising solutions for generations of new classes of miniaturizes membrane- less biofuel cells. The work now in progress is aimed at detail characterization of the interfacial electron-transfer rates, analysis of biocatalytic rate constants and understanding the mechanism of the direct electron transfer between GOx and electrode surface. Our research will be also directed at optimization such parameters as reproducibility and life time of the GOx electrode.[3]

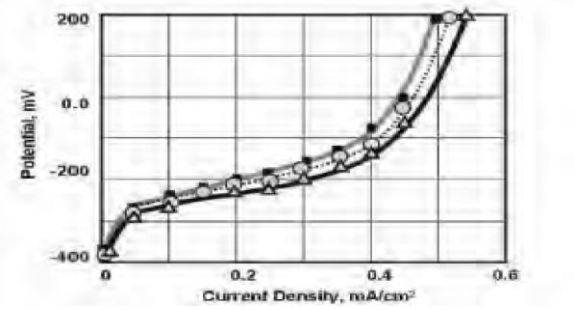

Figure 4. Polarization curves of three TP/MWNTs/PEI, GOx, Nafion electrodes. 20 mM phosphate buffer, pH 6.9, 0.1M KCl, 20 mM glucose 20 C. 0.1 cm2 electrodes.

5. Conclusion

When the electrode was coupled with a LAC-hPG electrode as acathode, a peak power density of 6 μW cm^{-2}at 0.2 V vs SCE wasachieved. Future work will be regarding the implementation of the enzyme hPG electrodes in a micro biofuel cell for healthcare applications. For development effective and reliable glucose oxidase anode for biofuel cell it is important to understand the mechanism of direct electron transfer between GOx and electrode surface. Many papers related to this subject indicate that application of nanometer scale and well-graphitized structure of CNT particles facilitates DET between active site of GOx and electrode surface. It can be hypothesized that the highly porous three-dimensional carbon nanotube network on the microelectrode surface has probably working as ‘‘nanowire’’ around enzyme molecule and promotes the electron transfer between enzyme and electrode surface. Therefore, the proposed immobilization strategy as well as materials and characterization tool provide a promising platform for constructing EFCs fed with glucose.

Acknowledgments

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