Biosensors and Bioelectronics 97 (2017) 46–52

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Transparent, mediator- and membrane-free enzymatic fuel cell based on nanostructured chemically modified indium tin oxide electrodes

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ARTICLE INFO

Keywords:
Indium tin oxide
Nanoparticle
Membrane-free
Mediator-free
Transparent enzymatic fuel cell

ABSTRACT

We detail a mediator- and membrane-free enzymatic glucose/oxygen biofuel cell based on transparent and nanostructured conducting supports. Chemically modified indium tin oxide nanoparticle modified electrodes were used to substantially increase the active surface area without significantly compromising transparency. Two different procedures for surface nanostructuring were employed, *viz.* spray-coating and drop-coating. The spray-coated biodevice showed superior characteristics as compared to the drop-coated enzymatic fuel cell, as a result of the higher nanostructured surface area as confirmed by electrochemical characterisation, as well as scanning electron and atomic force microscopy. Subsequent chemical modification with silanes, followed by the immobilisation of either cellobiose dehydrogenase from *Corynascus thermophiles* or bilirubin oxidase from *Myrothecium verrucaria*, were performed to obtain the bioanodes and biocathodes, respectively. The optimised biodevice exhibited an OCV of 0.67 V and power output of up to 1.4 μW/cm^2 at an operating voltage of 0.35 V. This is considered a significant step forward in the field of glucose/oxygen membrane- and mediator-free, transparent enzymatic fuel cells.

1. Introduction

Biological fuel cells (BFCs) in general, and enzymatic fuel cells (EFCs) in particular, have been envisioned as electrical power sources for self-contained bioelectronics including biomedical devices operating *in vivo* and *ex vivo* (Castorena-Gonzalez et al., 2013; Cosnier et al., 2016, 2014; Falk et al., 2013b, 2014; Halámková et al., 2012; Heller, 2004; Katz, 2014; Leech et al., 2012; Rasmussen et al., 2016; Shleev, 2017).

For certain applications, *e.g.* wearable electronics (Bandodkar and Wang, 2014, 2016; Pankratov et al., 2016) and biosolar cells (Somasundaran et al., 2011), the transparency of the EFC should be taken into account. The use of transparent conductive nanomaterials allows improving the aesthetic and cosmetic issues that are relevant in the case of smart electronic contact lenses (Blum et al., 2014; Parviz, 2009) and body-attachable wearable electronic platforms (Kim et al., 2015; Trung et al., 2016).

The electric power is generated by converting *in situ* available chemical energy from different biofuels present in human tears into electric energy. Several possible biofuels are present in lachrymal liquid, *e.g.* glucose, lactate, ascorbate and pyruvate, as well as neurotransmitters, although they appear in limited supply, while a biooxidant, molecular oxygen (O_2), dissolved in tear fluid is abundantly available (Pankratov et al., 2016). The very first publications detailing BFCs as power sources for electronic contact lenses appeared in 2012–2013: nanostructured glucose/oxygen (Falk et al., 2012) and ascorbate/oxygen (Falk et al., 2013a) BFCs based on direct electron transfer (DET) reactions were fabricated and tested in human lachrymal liquid.

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http://dx.doi.org/10.1016/j.bios.2017.05.040
Received 8 April 2017; Received in revised form 18 May 2017; Accepted 22 May 2017
Available online 23 May 2017
0956-5663/

Despite a need for transparent biodevices, to the best of our knowledge, there are only two reports in the literature regarding transparent EFCs (Amao and Takeuchi, 2008; Pankratov et al., 2015). Among the transparent and conducting supports indium tin oxide (ITO) is the most commonly employed (Gilstrap et al., 2008) in transparent electrochemical devices, since it presents a high optical transparency and conductivity (Dattoli and Lu, 2011). ITO also offers several other attractive physical properties, *e.g.* high stability under physiological conditions (Yang and Li, 2005). Despite the attractive properties of ITO, to the best of our knowledge, there are no reports in the literature regarding EFCs fabricated using ITO surfaces nanostructured with indium tin oxide nanoparticles (ITO NPs). Below we detail the very first transparent, mediator- and membrane-less EFCs based on nanostructured chemically modified ITO electrodes with covalently attached anodic and cathodic redox enzymes.

2. Material and methods

2.1. Reagents

N-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), ethanol (absolute), (3-glycidyloxypropyl)trimethoxysilane (GLYMO), ≥ 98%, Indium tin oxide (ITO) conductive glasses (15–25 Ω/sq), methanol and *N*-hydroxysuccinimide (NHS), 98% were purchased from Sigma-Aldrich (Steinheim, Germany). Acetic acid, dichloromethane, p.a., potassium chloride, sodium bicarbonate, and sodium hydroxide were acquired from J.T. Baker (Deventer, The Netherlands). Argon (Ar, 99.998%) and O_2 (> 99.5%) were obtained from Air Liquide (Düsseldorf, Germany), disodium phosphate, monopotassium phosphate, sodium acetate, 99.0% and toluene were from VWR-Chemicals (Langenfeld, Germany), D(+)-glucose, monohydrate and TRIS, ultrapure, were from AppliChem (Darmstadt, Germany), (3-aminopropyl)triethoxysilane (APTES), 99% was from ABCR (Karlsruhe, Germany), ITO nanoparticles (ITO NPs, product number 4111CB, 99.99%, 20–70 nm) were from Skyspring Nanomat. (Texas, USA), sodium chloride, 99.5% was from Carl Roth (Karlsruhe, Germany), sodium carbonate from Merck (Darmstadt, Germany), and 2-(N-morpholino)ethanesulfonic acid (MES) from Biomol (Hamburg, Germany).

2.2. Enzymes

Cellobiose dehydrogenase from *Corynascus thermophilus* (CDH) expressed in *Pichia pastoris* was obtained as previously described (Coman et al., 2010) and used for the fabrication of the transparent ITO bioanodes. In the initial aliquot the enzyme was dissolved in 50 mM sodium acetate buffer, pH 5.5 in a concentration of 25 mg/mL. An aliquot of 3.61 mg/mL of *Myrothecium verrucaria* bilirubin oxidase (BOx) was used for the fabrication of the ITO biocathodes. The enzyme was produced recombinantly in *Aspergillus oryzae*, as previously published (Xu et al., 1996), purified using a previously reported protocol (Falk et al., 2013a), and stored in 20 mM tris buffer, 100 mM Na_2SO_4, pH 8.

2.3. Nanostructuring process of ITO coated glass electrodes

ITO conductive glasses were cut into chips of 10 mm × 25 mm × 1.1 mm. The following cleaning procedure involved sonication in ethanol and subsequently in methylene chloride, 15 min each and additionally storing in ultrapure water for 20 min. After this, a working area of 10 mm × 10 mm was selected for electrode nanostructuring (Fig. 1).

Dispersions with different percentages (~ 3 wt% and ~ 15 wt%) of ITO NPs in methanol were prepared for the nanostructuring process aiming on two different mass loadings (0.25 mg/cm^2 and 1.25 mg/cm^2) on the surface of ITO thin film coated glasses. The dispersions were immersed in an ultrasonic bath for 20 min for an efficient homogenisation. Afterwards, for fabrication of the drop-coated ITO NP electrodes, 10 µL of the dispersion were dropped on the selected working area of the ITO coated glasses. After drying in air, the nanostructured surfaces were employed for chemical modification.

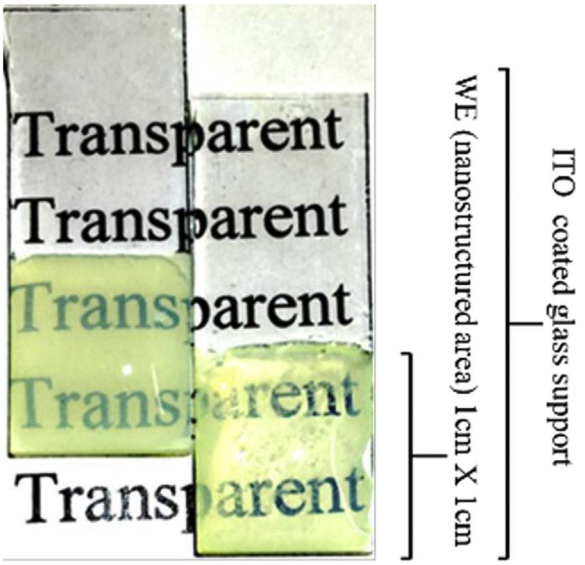

Fig. 1. Photographic images of ITO nanoparticle spray-coated (left) and drop-coated electrode (right).

In parallel, to fabricate the spray-coated electrodes, ITO thin film coated glasses were fixed on a solid copper heating block and heated up to 70 °C. While stirring the sample, a suspension of ITO NPs (2 mg/mL in methanol) was sprayed in a step by step procedure onto the electrode surface with the help of a compressed air nozzle. Thereby, the spray head was moved in an array of 1.2 cm × 1.2 cm with a fixed z-height above each electrode. This process was repeated several times, until the desired electrode loadings (0.25 mg/cm^2 and 1.25 mg/cm^2) were reached. Parameters and further information can be found in Supporting information (SI), *viz.* in SI Table S1 and Fig. S1.

2.4. Evaluation of the transparency

Quantitative transparency measurements employing UV–vis spectroscopy in air were performed with a set of enzyme-free modified ITO NP drop-coated electrodes, related to bare ITO quartz glass electrodes. A Cary® 50 UV–vis spectrophotometer from Varian (Utrecht, The Netherlands) was used for that purpose. Absorbance values for five prepared electrodes for each mass loading were measured in the wavelength range between 200 and 1100 nm and averaged. Transmittance values were calculated by using the logarithmic relation between these quantities.

2.5. Chemical modification of electrodes for enzyme immobilisation

Chemical modification of the nanostructured surfaces was optimised for each biocatalyst. For the chemical modification of the anodes, the cleaned ITO glasses were immersed in toluene with 1% (*v*/*v*) APTES overnight and stirred at room temperature. To carry out the chemical modification of cathodes, the cleaned ITO glasses were immersed for 20 min under stirring in toluene with 0.1% (*v*/*v*) GLYMO at 70 °C. The silanised ITO glasses were then rinsed with toluene in both cases.

2.6. Enzyme immobilisation

For CDH immobilisation, the enzyme solution was diluted in the