Glucose microfluidic fuel cell using air as oxidant

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A B S T R A C T

A bioanode was constructed using glucose oxidase enzyme (GOx) supported on multi-walled-carbon nanotubes (MWCNTs) in the presence of glutaraldehyde (GA) (GOx/MWCNTs-GA) and evaluated in an air-breathing hybrid glucose microfluidic fuel cell (HG-μFC). The air-breathing HG-μFC operated under physiological conditions (5 mM glucose at pH 7 with an air-exposed cathode) delivers an open circuit value of 0.72 V with 610 mW cm⁻² of maximum power density, and shows potential possibilities to develop future implantable applications.

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Introduction

Glucose fuel cells (GFCs) are a type of conventional fuel cells that oxidize glucose in the anode and reduce oxygen in the cathode to generate electric energy. The GFCs, depending on the type of catalyst used, can be classified into different subtypes: abiotic, microbial and enzymatic [1]. Enzymatic glucose fuel cells (EGFCs) are attractive due to their high specificity for β-D-glucose, production of power densities in the order of several mW cm⁻², relatively inexpensive production and ability to work under conditions similar to physiological conditions (37 °C and pH 7) [2]. These qualities make them very effective for use in devices such as pacemakers, defibrillators, cochlear implants, neuro-stimulators, artificial hearts and drug delivery systems in living organisms [3]. However, EGFCs can produce low current density and power due to the poor stability associated with improper immobilization, inefficient electron conduction and the lifetime of the enzyme [4,5]. A few studies have already reported the use of an abiotic electrode (anode or cathode) in an enzymatic glucose fuel cell with the main target of providing stability and higher performance [6,38]. Thereby, hybrid glucose fuel cells
Glucose oxidase (GOx) is the enzyme most employed as anode in EGFCs due to its stability, high catalytic activity and high selectivity toward glucose oxidation, as well as its low cost [8]. Many EGFCs that work involving GOx use PEMs (proton exchange membranes), which incorporate mediators to facilitate electron transfer from the enzyme to the electrode [9]. In this context, carbon nanotubes (CNTs) have been introduced to favour direct electron transfer because the specific nanowire morphology allows close interaction with the active site of the enzyme [10]. In addition, CNTs have unique properties such as high specific surface area, excellent biocompatibility, antifouling properties and high conductivity [11]. Furthermore, the PEM types of EGFCs are complexes and limit the possibility of micro fabrication; also, the PEM’s membrane tends to swell, shrink and deform by drying or dehydration, resulting in operating inefficiencies [12]. Therefore, the replacement of PEM with co-laminar fluid flow in microfluidic fuel cells (μFCs) provides a viable alternative working towards miniaturized power devices with small power requirements [13]. Advantages of employing μFCs are the increased rates of mass transfer, less time consumption, reduced waste stream volume and problems related with the membrane [14]. Further advantage related to the use of liquid fuels is their higher volumetric energy densities compared to gaseous fuels [1,6].

In this work, one type of hybrid glucose microfluidic fuel cell (HG-μFC) that uses an exposed-abiotic cathode (air-breathing) and a bioanode prepared by cross-linking glucose oxidase enzyme (GOx) over multiwalled-carbon nanotubes (MWCNTs) was evaluated. The air-breathing HG-μFC was operated in the presence of 5 mM glucose (concentration close to that found in blood) in phosphate buffer pH 7 as anolyte, delivering an open circuit potential value (OCP) of 0.72 V and 610 μW cm⁻² of maximum power density, which is a high value reported for this kind of device (Table 1).

### Table 1: Overview comparative of the HG-FC performances already reported.

<table>
<thead>
<tr>
<th>Electron transfer anode</th>
<th>Cathode (catholyte)</th>
<th>Anode (anolyte)</th>
<th>OCV (V)</th>
<th>P max/(μW·cm⁻²) (operating voltage)</th>
<th>Flow rate (μL·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anode: DET GOx/CNT-GA (Glu 200 mM) Pt (air-breathing)</td>
<td>Pt (gas diffusion electrode)</td>
<td>Glu (100 mM)</td>
<td>0.12</td>
<td>120 (0.1 V)</td>
<td>N.A</td>
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<tr>
<td>Anode: MET GOx/MWCNT-GA (Glu 200 mM) Pt (air-breathing)</td>
<td>Pt (gas diffusion electrode)</td>
<td>Glu (100 mM)</td>
<td>0.13</td>
<td>60 (0.1 V)</td>
<td>N.A</td>
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<td>Anode: DET GOx/MWCNT-GA (Glu 5 mM) Pt/C (air-breathing)</td>
<td>Pt (gas diffusion electrode)</td>
<td>Glu (100 mM)</td>
<td>0.48</td>
<td>105 (0.45 V)</td>
<td>1</td>
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<td>Anode: MET GOx/CNT-GA (Glu 200 mM) Pt-carbon (acetate buffer O₂ purging)</td>
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**Chemicals**

Glucose oxidase enzyme (GOx) from Aspergillus Niger (100,000–120,000 U/g) type X-S, D(-)-glucose ACS reagent and glutaraldehyde solution (GA) 50% were purchased from Sigma–Aldrich. KOH pellets (87%), H₂SO₄ (97.9%), HNO₃ (70%) and isopropyl alcohol were supplied by J. T. Baker. Nafton⁴ 5% solution was acquired from Electrochem Inc. Phosphate buffer solution (PBS) was prepared by using Na₂HPO₄ and KH₂PO₄ from J. T. Baker. All aqueous solutions were prepared using deionized water (p > 18 MΩ cm).

**Preparation of GOx/MWCNT-GA bioanode**

For the half-cell experiments, a graphite surface (GP, dimensions 0.69 x 0.98 mm x 3 mm) was used as the working electrode. GP electrode was first polished with alumina (0.05 μm) until a mirror finish was obtained and then washed in an ultrasonic bath in acetone and deionized water for

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(HGFC) represent an attractive alternative to be fabricated for these purposes [7].
20 min. After, the GP electrode was subjected to an electrochemical pre-treatment procedure by cycling in a solution of 0.5 M H₂SO₄ between −0.9 V and 1.3 V at 100 mV s⁻¹. Subsequently, the GP was washed with deionized water and dried at room temperature. On the other hand, MWCNTs were cleaned in 9 M HNO₃ at reflux for 24 h and dispersed in isopropyl alcohol (1 mg mL⁻¹) under ultrasonication for 1 h before being mixed with a solution of GOx-GA that was previously prepared by incorporating 5 mg mL⁻¹ GOx in PBS pH 7 and 1% GA to immobilise via cross-linking. Finally, 50 μL of GOx/MWCNT-GA ink was deposited onto the GP surface and dried at room temperature.

**Characterization and electrochemical measurements**

The electrochemical tests in the half-cell consisted of cyclic voltammetry performed by potentiostat/galvanostat (BioLogic SAS Science Instrument VSP) in a standard three-electrode glass cell using a saturated calomel electrode (SCE) and Pt wire as the reference and counter electrode, respectively in N₂-saturated 0.1 mM PBS and room temperature. Additionally, Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR) spectra were collected using a Perking Elmer FTIR spectrophotometer (Precisely Spectrum), in the spectral range of 600–4000 cm⁻¹ for the ATR.

**Hybrid glucose microfluidic fuel cell setup**

The air-breathing HG-μFC consisted of one microchannel sandwiched between a pressure sensitive adhesive material and a piece of 20 micron-thick micro-porous Toray® carbon paper (Technoquip Co Inc TGP-H-120) as the current collector (See Scheme 1). The fabrication was performed as follows: a silicone polymer film Silastic® (3-mm-thick) micromachined with a cutting plotter (Graphthe America Inc.) of 3.0 long × 1.0 cm wide and an opening of 0.1 × 0.9 cm was used as the microchannel. The anodic current collector was fabricated by using a material ARcare® 8890 (Adhesives Research Inc.) [6] covered by 0.66 cm² of adhesive graphite paper; meanwhile Toray® carbon paper (ElectroChem, Inc.) was used as the cathodic current collector. Dimensions of both current collectors were 3.0 × 1.0 cm wide with a central area of 0.95 × 0.47 cm where the catalyst was placed. The device was assembled for tests by aligning the three components and placing them between two polymethylmethacrylate PMMA pieces that were tightened by screws. In this case, the inlet solutions consisted of PBS (pH 7) and 5 mM glucose in N₂-saturated PBS for the cathodic and anodic compartment, respectively. The fuel cell tests were performed using a pressure driven fluid rate of 0.5 and 1.5 mL h⁻¹ for the anolyte and catholyte, respectively. The flows were electronically controlled using a syringe pump (NE-400, New Era Pump Systems Inc.). The current and power

![Scheme 1 – Schematic representation of the air-breathing hybrid glucose microfluidic fuel cell configuration.](image-url)
densities reported for this microfluidic fuel cell were calculated according to the planar active area of the electrodes in the microchannel (0.09 cm²). The cathode was covered with a catalytic ink containing Pt/V XC-72 (40% from E-TEK) 7 μL of Nafion® (5% H₂O) and 73 μL of isopropyl alcohol, which was deposited by spray technique on the surface with a final metal loading of 1 mg cm⁻² over the entire surface. In the bioanode, the GOx/MWCNT-GA catalyst was dropped on the surface.

Results and discussion

Characterization of GOx/MWCNT-GA anode electrode

Fig. 1a compares the electrochemical responses of GP and GP modified with GOx/MWCNT-GA electrodes in PBS (pH 7), which exhibits a pair of anodic and cathodic peaks located at −0.46 and −0.505 V, respectively, which are attributed to the FAD/FADH₂ redox of the electroactive centre of GOx [16]. The difference of the potential peak of the process (ΔEp) is approximately 45 mV and a formal potential (E⁰) of 0.48 V vs. SCE; these values are similar to the pair of FAD/FADH₂ peaks already reported at pH 7.0 and 25.8°C [17]. Both potential values suggested an efficient electron transfer between the GOx assisted by the MWCNTs on the GP surface [18].

Additionally, the electrocatalysis activity of GOx/MWCNT-GA was evaluated in N₂-saturated PBS solution pH 7 containing 5 mM K₃Fe(CN)₆ as redox mediator in the absence and presence of 5 mM glucose. In Fig. 1b well-defined redox peaks of K₃Fe(CN)₆ can be observed; however in the presence of glucose, the current density increases slightly suggesting that the enzyme is active [21].

Additionally, the effect of different scan rate values in an interval between 20 and 200 mV s⁻¹ on GOx/MWCNT-GA on
GP in PBS (pH 7) was tested (Fig. 2). A linear increasing of both anodic and cathodic peaks was observed as a function of the scan rate. This result indicates that the electron transfer process occurring at the GOx/MWCNT-GA film on the GP surface is controlled by an electrochemical process [22]. On the other hand, the electron transfer rate constant (Ks) for GOx/MWCNT-GA on GP was estimated according to the model of Laviron for a surface-control electrode process, considering a charge transfer coefficient of $\alpha = 0.5$ [23]. The obtained Ks value is 1.011 s$^{-1}$ which is similar to values already reported using MWCNTs in enzymatic electrode arrangements [24,25].

It is well known that the electrochemical response of GOx immobilized on a surface is due to a redox reaction of FAD/FADH$_2$ bound to the enzyme, which transfers two electrons coupled with a two proton redox reaction [26]. Fig. 3 shows that an increase of the pH of the solution leads to a negative shift of potential for both anodic and cathodic peaks. The slope for a linear plot of $E_0$ vs pH is $-55$ mV pH$^{-1}$ ($r = 0.9983$), which is close to the theoretical value of $-58.6$ mV pH$^{-1}$ at 22 $^\circ$C for a reversible system, indicating that two protons and two electrons participate in the electron transfer in the electrochemical reaction of GOx/MWCNT-GA immobilized onto the electrode carbon [27].

**FTIR characterization of GOx/MWCNT-GA**

Fourier transform infrared spectra at a range wave number 600 at 4000 cm$^{-1}$ for GOx mixed with MWCNT-GA were recorded, analysed and compared with pure GOx. IR spectra for both are presented in Fig. 4. The FTIR spectra of pure GOx shows two characteristic absorption peaks at 1641 and 1537 cm$^{-1}$ of the amides I and II, respectively. The peak observed at 1641 cm$^{-1}$ is attributed to the C=O of the peptide linkage in the protein backbone, and the vibration at 1537 cm$^{-1}$ is related to the combination of the N–H in plane bending and the C–N stretching of the peptide groups [28,29]. The FTIR spectra of GOx/MWCNT-GA show identical vibrations with respect to pure GOx indicating the presence of GOx in the MWCNT-GA mixture.

**Performance of an air-breathing hybrid glucose microfluidic fuel cell**

The polarization and power density curves of the air-breathing HG-µFC operates under physiological conditions pH 7 and 5 mmol L$^{-1}$ glucose [30] are presented in Fig. 5 where an open circuit potential (OCP) of 0.72 V and a maximum power and current density of 610 µWcm$^{-2}$ and 2.45 mA cm$^{-2}$ respectively are observed. The polarization curve shows an increase in the ohmic loss and the variation of the cell potential corresponding to zero current. This phenomenon could be attributed to the effect of the availability of oxygen (0.2 cm$^2$ s$^{-1}$ diffusion coefficient of oxygen in air) [15], delivery directly from air-exposed electrode in the cathode [31]. The performance of the HG-µFC that works with an air-exposed cathode achieved the highest value reported to date for hybrid glucose microfluidic fuel cells that use GOx/MWCNT. Additionally, an HG-µFC in a closed configuration (data not
shown) operated under the same conditions, except the O2-saturated catholyte, was evaluated as comparison. The closed device delivers the similar OCP value, but the maximum current and power density decreased around 50%. This result can be attributed to limited oxygen concentration in the cathode due to the oxygen diffusion coefficient in aqueous media (2 x 10^-5 cm^3 s^-1) which is lower compared to that value in air [31].

Furthermore, the air-breathing HG-μFC was maintained under continuous operation for around 150 min, discharging the device each 25 min. Fig. 6 shows that the OCP value decreased from 0.72 V until 0.62 V, meanwhile the current density decreased to 2.25 mA cm^-2 resulting in a final maximum power density value of 565 µW cm^-2 after 150 min of continuous operation. This result is related with the capacity of the recovery and stability of the device.

Table 1 summarizes different types of HG-FC already described in the literature that have used GOx wiring MWCNTs in the anode and Pt in the cathode or abiotic and utilized mediator electron transfer. All of the devices are PEM type design but did not work under a microfluidic regime.

Finally, the fuel utilization (FU), which is defined as the current output divided by the flux of reactant entering the channel, is estimated by the following equation:

\[ \text{FU} = \frac{1}{n\text{FCQ}} \]

where \( n \) is number of exchanged electrons, \( F \) is Faraday’s constant, and \( C \) is the inlet concentration. 1.5%FU is obtained for the air-breathing HG-μFC at low flow rate (8.33 µL min^-1). According to Zebda et al. the typical fuel utilization for glucose microfluidic fuel cell is 1% when the device is operated from 100 to 1000 µL min^-1 flow rate [40].

**Conclusions**

An enzymatic bioanode was constructed by means of immobilization of glucose oxidase enzyme on MWCNT in the presence of glutaraldehyde and evaluated toward glucose oxidation reaction in an air-breathing hybrid glucose microfluidic fuel cell. The comparative analysis of FTIR spectroscopy and electrochemical tests of pure GOx versus GOx-MWCNT-GA electrode confirms the presence of the GOx enzyme on the surface of the electrode. The performance of GOx/MWCNT-GA and Pt/C XC-72 as bioanode and cathode in the air-breathing HG-μFC evaluated under physiological conditions delivers an OCP of 0.72 V and a 610 µW cm^-2 of maximum power density, which is the best result obtained to date for a glucose microfluidic fuel cell that uses an enzymatic anode and an air-exposed abiotic cathode. These results show the potential possibilities to develop future portable applications where it is necessary to use autonomous energy.

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