

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

Electronic Supplementary Information

Hydrogen peroxide produced by glucose oxidase affects the performance of laccase cathodes in glucose/oxygen fuel cells: FAD-dependent glucose dehydrogenase as a replacement

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Supplementary Figures

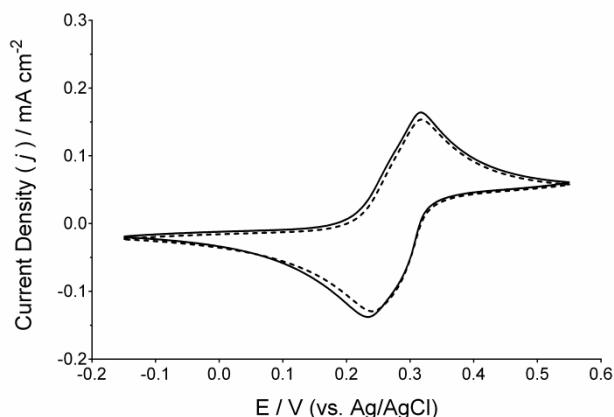


Fig. S1 Cyclic voltammograms of a BSA/Fc-LPEI control anode in a hydrostatic yet quiescent citrate buffer solution (0.2 M, pH = 5.5) containing 0 mM (solid line) and 200 mM (dashed line) glucose at a scan rate of 10 mV s⁻¹.

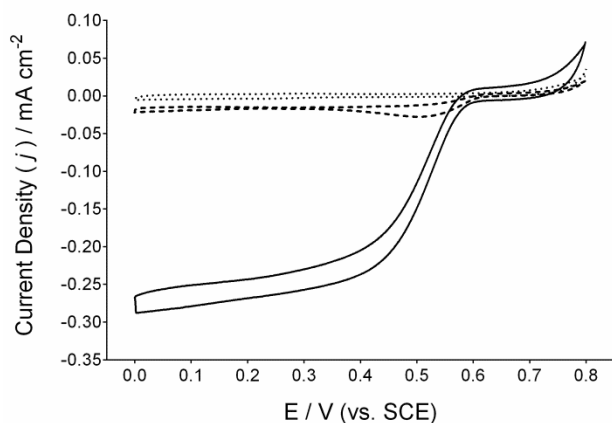


Fig. S2 Cyclic voltammograms of Toray paper cathodes incorporating Ac-MWCNTs, in a hydrostatic, quiescent citrate buffer solution (0.2 M, pH = 5.5, scan rate = 1 mV s⁻¹) operating in dissolved oxygen (solid line) and nitrogen-purged solutions (dashed line). Control experiments where laccase was substituted with BSA were operated in a quiescent solution (dotted line).

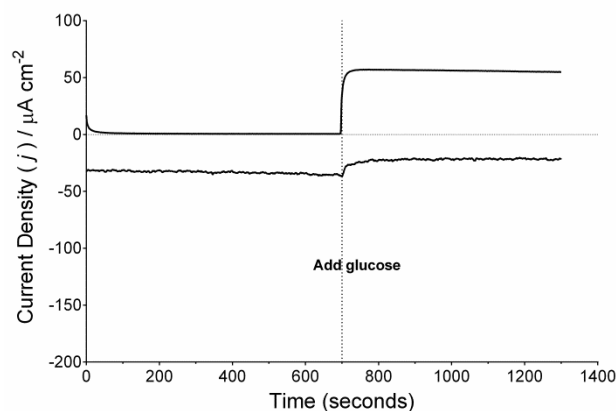


Fig. S3 Two simultaneous current vs. time responses of a GOx/Fc-LPEI anode vs. a laccase cathode in the same electrolyte, independently connected to their own counter (Pt mesh) and reference (SCE) electrodes. Potentials of 0.2 V (vs. SCE) and 0.41 V (vs. SCE) were applied at the cathode and anodes, respectively (where 0.41 V (vs. SCE) is the oxidation peak potential of Fc-LPEI + 0.05 V (as determined by cyclic voltammetry at a scan rate of 10 mV s⁻¹)). Starting electrolyte solutions were hydrodynamic (gentle stirring), nitrogen-purged citrate buffer solutions (0.2 M, pH = 5.5). At $t = 700$ seconds a glucose addition (from a nitrogen-purged solution) was made, giving a final glucose concentration of 200 mM.

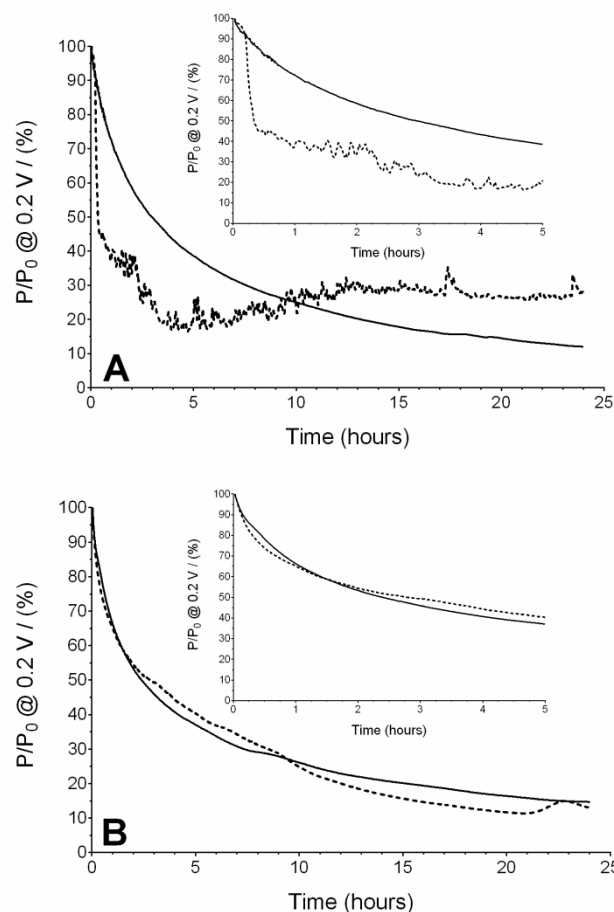
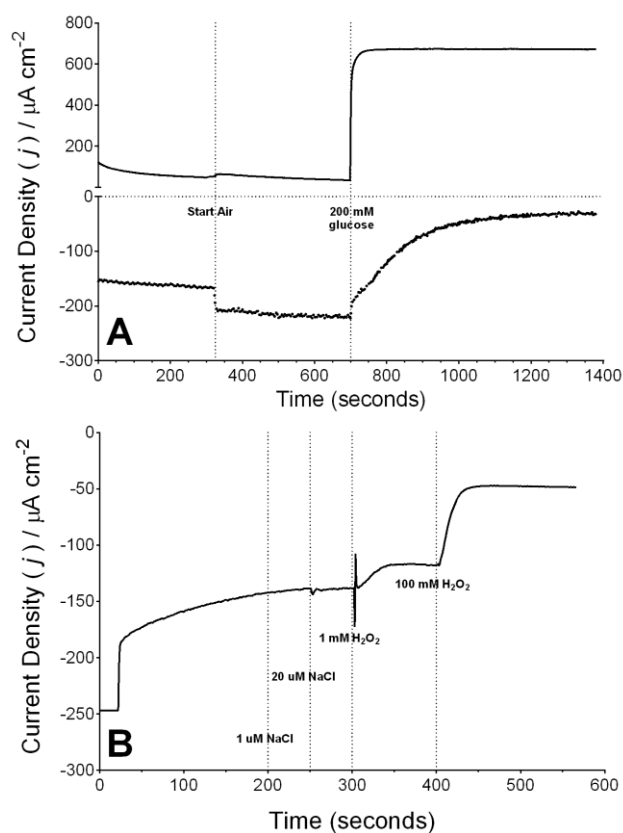


Fig. S4 (A) Two simultaneous current vs. time responses of FAD-GDH/Fc-LPEI electrodes (solid lines) vs. laccase electrodes (dashed lines) immersed in the same electrolyte, independently connected to their own counter (Pt mesh) and reference (SCE) electrodes. Potentials of 0.41 and 0.2 V (vs. SCE) were applied at the glucose oxidizing and O₂ reducing electrodes. At $t = 325$ seconds, gentle air-bubbling was introduced into the electrolyte (continuous until termination). At $t = 700$ seconds a single glucose injection was made giving a final glucose concentration of 200 mM. Starting electrolyte solutions were hydrodynamic (gentle stirring) yet quiescent citrate buffer solutions (0.2 M, pH = 5.5). (B) Current vs. time curve for a laccase cathode in a hydrodynamic, quiescent citrate buffer solution (0.2 M, pH = 5.5). Additions of NaCl were made at $t = 200$ and $t = 250$ seconds, giving final NaCl concentrations of 1 and 20 μM . Additions of H₂O₂ were made at $t = 300$ and $t = 400$ seconds, giving final H₂O₂ concentrations of 1 and 100 mM.

Fig. S5 (A) Average 24-hour stability curves for 3 replicates of FAD-GDH (solid line) and GOx (dashed line) BFCs coupled with laccase cathodes. Anodes were prepared on GC electrodes and cathodes were prepared on Toray paper. BFCs were operated in potentiostatic mode at a potential difference of 0.2 V, in hydrostatic yet quiescent solutions of citrate buffer (0.2 M, pH = 5.5) containing 200 mM glucose. The inset of (A) presents the same data, but scaled to an operation period of 5 hours. (B) Average 24 hour-stability curves for 3 repeats of FAD-GDH (solid line) and GOx (dashed line) BFCs coupled with Pt/C air-breathing cathodes. Anodes were prepared on GC electrodes. BFCs were operated in potentiostatic mode at a potential difference of 0.2 V, in hydrostatic yet quiescent solutions of citrate buffer (0.2 M, pH = 5.5) containing 200 mM glucose. The inset of (B) presents the same data, but scaled to an operation period of 5 hours.

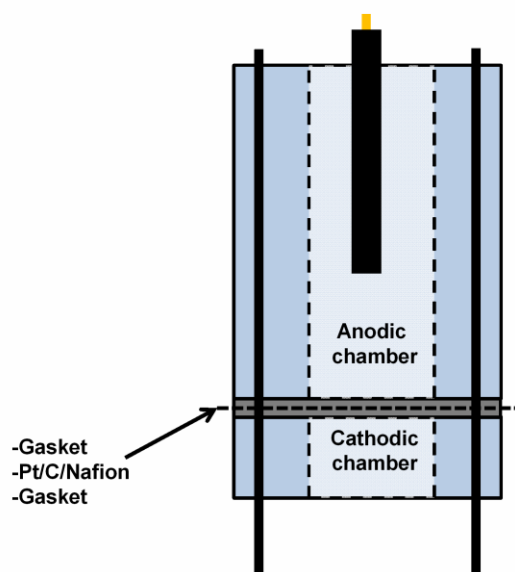


Fig. S6 Diagram of a typical fuel cell device containing a suspended glassy carbon-based anode vs. a Pt/C air-breathing cathode, where the anodic chamber is separated from the cathodic chamber by a Nafion® 212 proton-exchange membrane. GC geometric surface areas are calculated to be 0.071 cm² and cathodic (Pt/C) geometric surface areas are calculated to be 4.9 cm².

Experimental Procedures

GDH/Fc-LPEI and GOx/Fc-LPEI Anode Fabrication

Stock solutions of Fc-C₆-linearpolyethyleneimine (Fc-LPEI, 12 mg/ml, previously prepared¹), ethylene glycol diglycidyl ether (EGDGE, 10% v/v) and enzyme (either FAD-dependent glucose dehydrogenase (FAD-GDH) or glucose oxidase (GOx), 13 mg/ml) were prepared in water. Aqueous MWCNTs dispersions were prepared by the sonication of partially oxidised Nanocyl MWCNTs in water (0.1 mg/ml) for 2 minutes. The dispersions were left to stand for 24 hours prior to use. Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system.

Fc-LPEI (60 µL), EGDGE (3 µL), MWCNTs (10 µL) and either FAD-GDH or GOx (8.58 µL) were pipette-mixed in a small vial. 10 µL of this solution was added to the surface of a previously polished (and dried) glassy-carbon (GC) electrode and dried under a gentle positive air-flow. Once dried, the electrodes were left to stand overnight at room temperature and rinsed with water prior to use. For scaled-up electrodes (on Toray paper), 50 µL of the solution was used for an electrode with a geometric surface area of 1 cm².

Laccase Cathode Fabrication

A stock solution of tetrabutylammonium bromide-modified Nafion® (TBAB-Nafion®) in ethanol and anthracene-modified MWCNTs (Ac-MWCNTs) were prepared as previously reported.² Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system.

1.5 mg of crude laccase added to 75 µL of citrate buffer (0.2 M, pH 5.5) and gently mixed until dissolved. This solution was then added to 7.5 mg of Ac-MWCNTs and mixed by a combination of vortex-mixing and gentle sonication for a total of

4 minutes and 1 minute, respectively. TBAB-Nafion® in ethanol (25 µL) was then added to the mixture and vortex-mixed for a further minute. This mixture was then evenly painted onto Toray paper electrodes (geometric surface area of 1 cm²) with approximately 30 µL being deposited on each electrode. The electrodes were then gently dried under a positive air-flow, and stored at 4°C until use.

Laccase Activity Assays

The specific enzymatic activity of crude laccase was calculated using 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the substrate, at pH 5.5 and room temperature. Spectrophotometric analysis was performed on a Thermo Scientific® Evolution 260 Bio UV-Visible Spectrophotometer. Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system.

Stock solutions of ABTS (0.54 mM in water) and laccase (0.1 mg/mL in water) were prepared, prior to use. 1.125 mL of citrate buffer (50 mM, pH 5.5), 0.125 mL of the laccase solution and 0.125 mL of the ABTS solution were added to a cuvette. The solutions were mixed by inversion of the cuvette and the absorbance was recorded at a wavelength of 420 nm for 3 minutes. Control experiments consisted of substituting the 0.125 mL enzyme fraction with 0.125 mL of water.

For activity assays incorporating catalase, the laccase solution was replaced with a solution comprised of laccase (0.1 mg/mL) and catalase (0.2 mg/mL). Lastly, laccase activity assays in the presence of catalase were not performed in solutions of 100 mM H₂O₂, due to large quantities of O₂ production (by catalase) which affects the readability of the absorbance values. All experiments were performed in triplicate.

FAD-GDH/GOx Activity Assays

The specific enzymatic activity of crude GOx and FAD-GDH was determined using 2,6-dichlorophenolindophenol (DCPIP) as the substrate, at pH 5.5 and room temperature. Spectrophotometric analysis was performed on a Thermo Scientific® Evolution 260 Bio UV-Visible Spectrophotometer. Water used was taken from a Millipore Type 1 (Ultrapure) Milli-Q system.

Stock solutions of DCPIP (0.7 mM in water), glucose (1M in water) and either FAD-GDH (1 µg/ml in water) or GOx (0.5 mg/mL of water) were prepared. 8.5 mL of citrate buffer (pH 5.5, 50 mM), 0.5 mL of the DCPIP solution and 1 mL of the glucose solution were mixed; 1.45 mL of this solution was added to a 2 mL conical cuvette (l = 1 cm). 0.05 mL of the enzyme solution (either FAD-GDH or GOx) was added to the cuvette and mixed by swirling. The absorbance of this solution was measured for 5 minutes, at a wavelength of 555 nm. The molar absorptivity of DCPIP was calculated to be 8.465 mM⁻¹/cm at a wavelength of 555 nm, by successive dilutions of DCPIP. All experiments were performed in triplicate.

Nafion® Pt/C Fuel Cell

Pt/C air-breathing cathodes on Nafion® 212 perfluorinated membrane were prepared by hot-pressing Pt/C cloth (60 % Pt supported on Vulcan XC-72, 0.5 mg cm⁻²) onto Nafion® perfluorinated membrane for 10 minutes under 10,000 lbs of

force at 140°C. Fig. S7 shows a typical Pt/C fuel cell setup, coupled with a GC anode.

References

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2. M. T. Meredith, M. Minson, D. Hickey, K. Artyushkova, D. T. Glatzhofer and S. D. Minteer, *ACS Catal.*, 2011, **1**, 1683-1690.