Published in final edited form as:

Biofabrication. 2013 September ; 5(3): 035009. doi:10.1088/1758-5082/5/3/035009.

Cross-linked Glucose Oxidase Clusters for Biofuel Cell Anode Catalysts

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Abstract

The efficient localization of increased levels of active enzymes onto conducting scaffolds is important for the development of enzyme-based biofuel cells. Crosslinked enzyme clusters (CEC) of glucose oxidase (GOx) constrained to functionalized carbon nanotubes (CEC-CNTs) were generated in order to evaluate the potential of using CECs for developing GOx-based bioanodes functioning via direct electron transfer from the GOx active site to the CNT scaffold. CEC-CNTs generated from several weight-to-weight ratios of GOx:CNT were examined for comparable catalytic activity to free GOx in solution, with CEC-CNTs generated from a 100% GOx solution displaying the greatest enzymatic activity. STEM analysis of CEC-CNTs generated from 100% GOx to CNT (wt/wt) ratios revealed CEC clusters of $\sim 78 \mu\text{m}^2$ localized that the CNT surface. Electrochemical analysis indicates that the enzyme is engaging in direct electron transfer, and BFCs generated using GOx CEC-CNT bioanodes were observed to have a peak power density of $\sim 180 \mu\text{W}\cdot\text{cm}^{-2}$. These data indicate that the generation of nano-to-micro sized active enzyme clusters is an attractive option for the design of enzyme-specific biofuel cell powered implantable devices.

Keywords

Glucose Oxidase; Cross-linked Enzyme Cluster; Biofuel Cell

1. Introduction

The conversion of (bio)chemical energy to electrical energy for use in a variety of applications has attracted significant research interest in recent years. In particular, the development of micro and nano-scale implantable devices and sensors requires energy sources and testing platforms that of equivalent scale, yet provides sufficient power and/or signal [1-8]. Enzyme-based bio-fuel cells (BFCs) are promising sources of power for these

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devices, where one harnesses the potential energy from enzymatically catalyzed reactions utilizing natural substrates and resulting in reaction products that are metabolically safe [7, 9-13]. The efficient localization of increased levels of active enzyme onto conducting scaffolds is important for the development of enzyme-based materials for biosensor and BFC applications.

Several enzymes have been studied as candidates for BFC anode and/or cathode development, both separately and in combination. The production or usage of electrons in the catalytic function of the enzyme is the central key to utilization as a BFC anode or cathode. For instance, the copper-containing laccase is often employed in BFC cathodes [9, 14-18], and other enzymes examined as candidates for BFC applications include alcohol dehydrogenase [7], fructose dehydrogenase [19, 20], aldose dehydrogenase [21], horseradish peroxidase [22], cellulase [11] and tyrosinase [23]. With respect to biosensors and BFC anodes, the most widely studied enzyme is glucose oxidase (GOx), which was initially used in glucose biosensors for diabetics but has found increased use in BFC applications [1, 2, 4, 9, 10, 24]. Glucose oxidase, a ~140 kDa FAD-containing homodimeric oxidoreductase (Figure 1) [25-27], produces 2 electrons through the oxidation of α -D-glucose by molecular oxygen to D-gluconolactone and hydrogen peroxide [25]. Numerous studies have demonstrated reasonable power outputs from GOx-based anodes [21, 28-31]; GOx has also been used in full enzymatic BFCs, including BFCs composed of (anode/cathode) GOx/laccase [15, 16], GOx/alcohol dehydrogenase [7] and GOx/polyphenol oxidase [12, 32].

Many GOx-based sensors and BFC anodes have been developed through the use of mediators that facilitate electron transfer from the redox centre of GOx to the electrode [21, 33-37]. More recently, based on evidence that redox species of mediators in the electrolyte solution can interfere with the transfer of electrons [38], GOx-based sensors and BFC anodes have been designed such that direct electron transfer (DET) occurs from GOx to a conducting scaffold. This is done in order to narrow the gap between the GOx enzymatic reaction center and electrode to improve electron transfer efficiency. Numerous inorganic materials have been explored as electrodes for GOx immobilization including numerous nanoparticles [1, 39], stainless steel [40], gold films [41, 42], carbon nanotubes [28, 30, 43-45], nanofibres [36, 46] and graphene [29, 47, 48], as well as directly on Nafion membranes [49] and glassy carbon electrodes [50].

In order to increase the power output from a GOx-based DET anode, the loading of GOx must be increased to provide sufficient electrons to the circuit. Several studies have investigated increasing the enzyme load at the electrode by physical or chemical immobilization. Two such examples are the dispersion of CNTs in a GOx solution [51] and the mechanical compression of a GOx/CNT mixture [16]; both studies demonstrate an altered CNT morphology coupled with GOx activity. An alternate approach to increasing GOx loading at the electrode is to cluster the enzyme through precipitation [29, 30, 52-56], building on the high loading capacity of CNTs due to their large surface area for the attachment of clusters of GOx (Figure 1). This method results in clusters of active enzyme at the CNT electrode surface, with initial attachment of a GOx monomer achieved through interaction of one of the protein's 15 lysine or 22 arginine residues [26] to the COOH-functionalized CNT. In the current report, we present a detailed characterization of the incorporation of GOx onto a COOH-functionalized CNT scaffold in the form of cross-linked enzyme clusters (CECs) for use as a GOx-based DET anode catalyst. Our studies employ an in-house purified GOx, unlike other studies which use commercially-sourced enzyme, and demonstrate that in-house purified GOx displays a less heterogeneous GOx starting material. GOx-containing CEC-CNTs are generated under physiologically amenable conditions, and generate appreciable power density ($180 \mu\text{W}\cdot\text{cm}^{-2}$) when tested in a working BFC.

2. Materials & Methods

2.1 GOx Purification

Glucose oxidase used in the current study was obtained from a commercial source (EMD Biosciences) or expressed in *Aspergillus niger* by fermentation under standard procedures (NBS Fermenter, 500 rpm, pH 6.0, 30 °C, 96 hours) [25]. Commercial GOx was used without further purification while expressed GOx was purified as follows: after 96 hours expression, the culture was centrifuged (4 °C, 15 min, 7,500 × g), and two volumes of 95 % ethanol were added to the supernatant to precipitate GOx. Further centrifugation (4 °C, 15 min, 10,000 × g) yielded a precipitate that was resolved in a PBS buffer that was subjected to super filtration (160 kDa MWCO) and anion exchange chromatography on DEAE Sepharose (GE Healthcare). GOx purity was monitored by 12.5% SDS-PAGE (Figure 2a), and the protein was lyophilized for storage prior to CEC generation.

2.2 GOx Immobilization for CEC Generation

Generation of CEC-CNTs from soluble GOx was adapted from previously reported methods [30]. Commercially pre-functionalized multiwalled carbon nanotubes (COOH-MWCNTs) of 20-30 nm in outer diameter were obtained from cheaptubes.com (Brattleboro, VT, USA). Pre-weighed COOH-MWCNTs were suspended in sodium acetate buffer (pH 7.4) and reacted with 100 mg·mL⁻¹ N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC; BioBasic) for 1.5 hours to generate amine-reactive esters. The amine-reactive esters enable cross linking with primary amine-containing residues of GOx, which effectively immobilizes the protein onto the MWCNTs. Ammonium sulphate (90% v/v) precipitation of GOx (2 hours, 4 °C) clustered the protein at the sites of immobilized GOx; incubation with 5% v/v glutaraldehyde (GA; 10 min, 4 °C) generated crosslinked enzyme clusters (CECs). Solvent removal was achieved through filtration on 0.45 µm disc membranes, and CEC-CNT nanocomposites were resuspended in PBS buffer (pH 7.4) and stored at 4 °C.

2.3 Enzymatic Analysis of GOx and CEC-CNTs

Enzymatic analysis of free and CEC-CNT constrained GOx was conducted based on the method of Woodward *et al.* [57]. Briefly, equal volumes of free GOx (at 0.5, 1.0 or 5.0 mg·mL⁻¹) or CEC-CNTs (at 80%, 100% or 200% GOx:CNT (wt/wt)) were combined with 2 mM ferrous sulfate in 50 mM sodium acetate (pH 4.0) and mixed by inversion. To this mixture D-glucose was added to a final concentration of 1.3 mM, and GOx activity was measured through the reduction of Fe³⁺ to Fe²⁺ by H₂O₂ via absorbance at 340 nm room temperature for 50 minutes. Assays were conducted in triplicate, average values plotted (Figure 2b) and used to determine the optimal GOx:CNT ratio for CEC-CNT generation.

2.4 Electrochemical Analysis of GOx CEC-CNTs

Electrochemical measurements were conducted on an Autolab electrochemical analyzer (PGSTAT30; Eco-Chemie BV) utilizing a glassy carbon working electrode (GCE), a platinum wire counter electrode and calomel reference electrode. In this configuration, 5.5 mg of GOx CEC-CNT nanocomposite (100% wt/wt GOx:CNT) or CNTs alone were added to 200 µL of a 0.1% Nafion solution and sonicated for uniformity. Ten microlitres of the test solution was then added to a clean GCE (area = 0.38 cm²) and allowed to air dry. Assembled electrodes were purged with phosphate buffer (pH 7.0), and measurements were conducted using a scan rate of 100 mV·s⁻¹; data analysis was performed using the Origin 8 software package.

2.5 Electron Microscopy

Filtered CEC-CNTs and COOH-MWCTs were resuspended in a minimal volume of toluene with sonication and pipetted onto copper grids coated with an ultrathin (3 nm) carbon film. Scanning transmission electron microscopic (STEM) images were collected at the Centre for Nanostructure Imaging at the University of Toronto in high angle annular dark field (HAADF) [58] mode on a Hitachi HD-2000 electron microscope, with an accelerating voltage of 200 kV.

2.4 Biofuel Cell Assembly and Testing

Catalyst-coated membranes (CCM) with 5 cm² active area were fabricated using GOx CEC-CNT and Pt/C catalyst samples for anode and cathode sides of Nafion-212 membrane, respectively, using a microspray method. The membrane was fixed in a home-made fixture to ensure anode and cathode catalyst layers were exactly on the same area of the membrane. The catalyst loadings on the anode and cathode sides were about 0.5 mg GOx CEC-CNT and 0.5 mg Pt cm⁻², respectively. The catalyst-coated Nafion membrane was vacuum dried at 40 °C for an hour before assembling it in the test cell. The gas diffusion layer (GDL) was prepared with a teflonized non-woven 7 mil carbon paper (P50T, Ballard Material Products Inc., Lowell, MA, USA) substrate. A slurry of graphitized carbon black grade PUREBLACK 205-110 carbon (Superior Graphite Co., Chicago, IL, USA) with PTFE (TE3859 Teflon suspension, DuPont) dispersion in a mixture of isopropanol and de-ionized water (80:20 volume ratio) was prepared by ultrasonication for 20 min followed by magnetic stirring for 2 hr. The micro-porous layer was fabricated by applying the slurry on the carbon paper by microspray. Subsequently, the carbon paper with micro-porous layer was heat treated by sintering at 350 °C in air for 1 hr. The carbon loading for the micro-porous layer was approximately 3 mg·cm⁻² and the PTFE content was 30 wt%. Membrane electrode assemblies (MEAs) were assembled by sandwiching the catalyst-coated membrane with the GDLs inside the BFC test cell (Fuel Cell Technologies). Gas sealing was carried out using silicone coated fabric materials (Performance Plastics, CF1007) at a uniform torque of 40 lb-in. Single cell fuel cell performance was evaluated using a Greenlight G40 Test Station (Hydrogenics Test Systems, Burnaby, Canada) at room temperature. The catalytic activity of GOx CEC-CNT composites were collected using glucose (200 mM) and oxygen as fuel and oxidant, respectively.

3. Results & Discussion

To increase the power output of enzyme-based BFCs, one needs to increase either the enzyme activity or the loading of active enzyme at the electrode surface, either separately or in combination. CECs provide a highly concentrated, reusable, and thermally stable enzyme aggregate at the electrode, generally requiring less material to construct in comparison to other entrapment methods [54-56]. We prepared active GOx containing bionanocomposites by an initial covalent attachment, followed by CEC formation through a sequential addition of ammonium sulfate followed by glutaraldehyde (GA) to the GOx-CNT suspension (Figure 1). Fabrication of the composite this way allows a monolayer of GOx to attach via covalent interaction with available carboxyl functionalities on the CNT in order to provide a surface on which cluster anchoring can be established. In addition, assembling BFC anodic materials in this fashion promotes DET from the GOx to the CNT scaffold by anchoring the enzyme directly to CNTs, thereby removing any redox mediator and reducing predicted mediator-related power losses.

In an effort to develop a cost-effective means of BFC assembly and testing, we examined both a commercial and in-house expressed and purified GOx source, and CEC-CNT nanocomposite generation was carried out on a weight-to-weight ratio expressed as a

percent of total CNTs. Both commercially sourced and in-house expressed GOx showed reasonably clean protein and the correct molecular weight (Figure 2a). However, in-house purified protein was deemed more reasonable for CEC generation due to sharper banding on SDS-PAGE. The sharper banding indicates a more homogeneous enzyme sample, likely due to a more defined carbohydrate component of the expressed GOx. GOx contains a significant carbohydrate component (10-17% by mass) that imparts significant thermal stability to the protein [59], however is antagonistic to DET as it acts as a barrier to extraction of electrons from the FAD cofactor to an external carrier [60]. Thus a more defined, or even minimal, carbohydrate content is desirable for GOx-based BFC anodes; we are currently exploring other GOx expression systems to reduce/remove the carbohydrate shell to increase DET in CEC-CNT based BFCs. To determine appropriate starting ratios of GOx and CNTs for CEC-CNT generation, the relative enzymatic activity of GOx in solution and in CECs from several GOx:CNT ratios were compared (Figure 2b). Interestingly, while one would consider that a 200% GOx CEC-CNT would produce higher overall enzymatic activity, the 100% GOx:CNT sample produced the highest resulting enzymatic activity (Figure 2b). Therefore, all subsequent CEC-CNTs used for BFC anode assembly and testing were generated from in-house purified GOx in a 100% wt/wt GOx:CNT ratio. Indeed, the enzymatic activity of GOx in the 100% CEC sample approached that of $5 \text{ mg}\cdot\text{mL}^{-1}$ free GOx while the 200% CEC-CNT sample was shown to be most similar to $1 \text{ mg}\cdot\text{mL}^{-1}$ free GOx (Figure 2b). One reasonable explanation for this is that the 200% GOx CECs are too large for effective penetration of the glucose substrate or electron transfer to the CNT scaffold. This would result in decreased overall enzymatic activity and adversely affecting the overall electrical output of CEC-containing BFCs; the overall number and size of GOx CECs affect the effective electrical output.

In order to confirm that GOx had clustered as CECs at the CNT surface, samples were subjected to STEM analysis (Figure 3). It can be seen that in comparison to CNTs alone (Figure 3a), samples subjected the CEC generation (100% wt/wt GOx:CNT) do indeed show sizeable protein clusters at the CNT surface (Figure 3b). Inspection of the CECs at the CNT surface indicates an average area of the cluster at approximately $78 \mu\text{m}^2$. While the depth of CECs were not directly measured, it can be reasoned that the CECs are not all uniform in depth based on the observation of differential brightness of the images, which is dependent on the amount of protein within the clusters themselves. Assuming an average CEC cross-section of $8 \mu\text{m}$, the CECs be $624 \mu\text{m}^3$ in volume. Given a predicted volume (protein only) of the GOx dimer as 168.17 nm^3 , each CEC would contain approximately 3.71×10^9 GOx dimers. It is conceivable that while the size of the CECs is substantial in comparison to that of the CNTs, an increased number of smaller CECs more uniformly distributed along CNT surface, similar to other studies of GOx localization onto electrode surfaces [16, 29, 30, 51-53], would be more effective for DET. The larger CECs observed may be a result of a lack of accessibility to individual CNTs by the protein either during the initial incubation with the GOx protein or due to the spontaneous clumping behaviour of the protein with itself upon ammonium sulfate precipitation. We are currently investigating more effective methods for CNT dispersion and GOx interaction in solution to increase CNT coverage by CECs, for instance through ultrasonication methods that have shown effective localization of GOx onto CNTs [61].

GOx-based CEC-CNTs were characterized with cyclic voltammetry to evaluate enzymatic redox activity within the CECs (Figure 4). Similar to other studies that have employed a generalized deposition of GOx in mixture with CNTs [28, 42, 43, 45, 62], the CNTs were observed to add a capacitance characteristic to the anode (Figure 4, dotted line). The cathodic and anodic peaks were observed at -0.462 V and -0.539 V (Figure 4, solid line), respectively, which are similar to other studies of GOx on CNTs [28, 45] and nanospheres [39]. The cathodic and anodic peak separation (E_p) is 0.077 V , and the ratio of the cathodic

and anodic peak heights (E_O) was observed to be $\sim 1 \pm 5\%$. The E_O of the CEC-CNTs is similar to previous reported CV data of GOx-CNT anodes [28, 45] and GOx on nanospheres [39], thereby ascribing the observed redox potentials to DET from GOx to the CNT scaffold. The E_p was, however, slightly wider than previous studies and is likely due to DET versus MET in the previous study [28]. The CEC-CNT cathodic peak was integrated to $0.13 \mu\text{C}$, which corresponds to approximately 4.1×10^{11} active GOx molecules actively engaging in DET at the CNT surface.

To assess the catalytic activity of GOx CEC-CNT composites for use in BFCs, fuel cell data were collected from a Membrane Electrode Assembly (MEA) using a CEC-CNT based anode and Pt cathode with 200 mM glucose and oxygen as fuel and oxidant, respectively, at room temperature. The peak power density observed was $\sim 180 \mu\text{W}\cdot\text{cm}^{-2}$ for the CEC-CNT nanocomposite containing 100 wt. % GOx (Figure 5). BFC power output was stable over a period of twelve hours at room temperature, however power outputs were observed to decrease when the BFC was tested at temperatures above 40°C . To assess the applicability of our approach to CEC-CNT generation for bioanodes, we compared the peak power density of the 100% GOx CEC-CNT to several other MET and DET-based BFCs (Table 1). The power output of the current 100% GOx CEC-CNTs was greater than that observed in a previous study of GOx clusters on CNTs [30], however less than a more recent study of GOx clusters on graphene [29] (Table 1). Both these previous studies employed 200 mM glucose as fuel and ascribed power outputs to DET from GOx [29, 30], thereby providing a direct comparison to the present system. To our knowledge, the highest power density to date for a GOx-based DET BFC ($1.25 \text{ mW}\cdot\text{cm}^{-2}$) was shown for a BFC composed of a GOx/Catalase-CNT bioanode and Laccase-CNT biocathode where both the anode and cathode were generated using mechanical compression methods [16]. When compared to BFCs employing MET strategies, the peak power density output of the current BFC using a 100% GOx CEC-CNT bioanode is greater than other studies of GOx bioanodes [21, 32, 35, 37, 63] (Table 1). The highest MET-based power density output of a non-clustered GOx bioanode is $350 \mu\text{W}\cdot\text{cm}^{-2}$ for *A. niger* GOx [64] and $280 \mu\text{W}\cdot\text{cm}^{-2}$ using *Penicillium pinophilum* GOx [65]. Interestingly, the power density observed from the *P. pinophilum* GOx bioanode [65] was achieved using 5 mM glucose, which is close to the mean normal concentration of glucose in the blood [66]. While our *A. niger* GOx CEC-CNT demonstrated $180 \mu\text{W}\cdot\text{cm}^{-2}$ at 200 mM glucose, it is possible that power densities could be increased at more metabolically relevant glucose concentrations employing CECs generated from *P. pinophilum* GOx. We are currently investigating the potential of generating *P. pinophilum* GOx-CECs for BFC fabrication.

The primary benefit of creating CECs for BFC anode development is the mass incorporation of free GOx molecules at the CNT interface. The higher GOx content theoretically results in greater power densities that can be achieved through involvement of more GOx in the catalytic process, and promotes DET from the GOx active site to the CNTs to shuttle the liberated electrons for power generation. STEM analysis of our CEC-CNTs (Figure 3) indicate that we are indeed achieving increased loading of the enzyme at the CNT surface, with an approximate 3.71×10^9 GOx dimers per CEC; the GOx within these CECs are redox active (Figure 4). In addition, the cross-linking of GOx into CNT-constrained CECs should impart increased protein stability within the BFC; Lopez-Gallego *et al.* [67] have shown that the multiple covalent attachment sites provided by glutaraldehyde in a GOx clustered structure is 400-fold more stable than the free enzyme. Taken together, the use of CECs as anode materials for BFCs should provide increased stability and active lifetimes, as is seen in other studies [29, 30], and it is reasonable to suggest that the crosslinked CEC structure immobilized on the CNT scaffold imparts structural stability to the protein; we are currently investigating this possibility.

A challenge with the current strategy of CEC generation lies in overcoming the distance that exists between the CNT framework and the redox centre of GOx in order to allow electron siphoning without disturbing the integrity of the molecule and facilitate electron transfer closer to the potential of the enzyme [68]. As with many redox proteins, the FAD-containing catalytic redox centre in native GOx is deeply embedded within the monomer with the only access being through a 1 nm² opening [26, 69], thereby removing it from direct communication with an electrode. In addition, the significant carbohydrate content of GOx has been shown to have an antagonistic effect to DET as it acts as a barrier to extraction of electrons from the FAD cofactor to an external carrier [60]. These structural considerations indicate that active enzymes within the CEC that are relatively far away from the CNT scaffold would have a decreased effective DET. Indeed, the observation that 100% GOx CEC-CNTs produce relatively large (78 µm²) structures (Figure 3) with enzymatic activity similar to that of 5 mg·mL⁻¹ free enzyme (Figure 2b), while CEC generated from 200 wt% GOx CEC show lower activity attests to the need for increased numbers of smaller CNT-constrained CECs for more effective power generation. However, MEAs generated from GOx CEC-CNTs exhibit a ~180 µW·cm⁻² peak power density when tested in a functioning BFC. This power output, which is similar to other studies of GOx clusters on CNTs [30] and graphene [29], can be attributed to DET events. While the generation of an increased number of smaller CECs will likely result in overall increased power outputs, the current data suggest that increasing power densities in BFCs through the generation of CECs is a facile means for the generation of enzyme-specific BFC anodes. In order to generate more compact CECs for increased DET, we are exploring the removal of the carbohydrate, by either enzymatic or recombinant methods, in addition to optimization of GOx immobilization strategies and CEC-CNT generation for increased BFC power densities. Micro sources of power for medical devices, both prosthetic and implantable, that use glucose as a fuel source is an area we are pursuing in our laboratories, and in this context the results reported herein are relevant. For such devices mediator-less electron transfer has significant advances.

Acknowledgments

The authors wish to thank Neil Coombs at The Centre for Nanostructure Imaging at the University of Toronto for assistance with STEM imaging, and Prof. Sylvie Morin and Dr. Muhammad F. Khanfar of the Department of Chemistry at York University for assistance with the electrochemical measurements of the GOx-CNTs, useful discussions and review of the manuscript. This work was supported by grants to GFA from the Natural Sciences & Engineering Council of Canada, the Canadian Foundation for Innovation and York University. AMK acknowledges funding from Arizona State University. VR and SV acknowledge the support from the Rothschild Foundation, NIH, and Children's Hospital, Boston.

Abbreviations

BFC	Biofuel cell
GOx	Glucose oxidase
CEC	Cross-linked enzyme cluster
DET	Direct Electron Transfer
MEA	Membrane Electrode Assembly

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Figure 1.

Schematic representation of the generation of glucose oxidase (GOx; PDB ID 1GPE) [26] containing CEC-CNT nanocomposites for incorporation in BFC as anode catalysts. Carboxyl-functionalized CNTs (CCOH-CNTs) are added to a solution containing GOx and CECs are generated through the sequential addition of ammonium sulfate and glutaraldehyde (GA) to the GOx-CNT solution. CEC-CNTs catalyst coated membranes are then utilized the bioanode inside BFC test cells with using a Pt cathode.

Figure 2.

Glucose oxidase sample purity and enzymatic analysis for CEC-CNT generation. (a) SDS-PAGE analysis of commercial (Lane 1) and in-house expressed/purified (Lane 3) GOx. While both samples show distinct banding at the appropriate molecular weight, in-house produced GOx shows a cleaner banding pattern indicative of a more homogeneous sample. Therefore, only in-house purified GOx was employed for GOx CEC-CNT generation. (b) Enzymatic analysis of free and CEC-CNT glucose oxidase. Relative activities of free GOx at several concentrations were compared with those of GOx based CEC-CNTs. CEC-CNTs generated from a 100% ratio of GOx to CNTs (*) was shown to be optimal with activity close to that of 5 mg·mL⁻¹ free enzyme (). Conversely, 200% GOx CECs (+) only demonstrated activity similar to that of a 1 mg·mL⁻¹ free enzyme solution. The data are the means and standard deviations of three replicates.

Figure 3. STEM characterization of CEC-CNT nanocomposites. STEM images of unstained CNTs (a) and CEC-CNTs (100% wt/wt GOx:CNT) (b) taken in high angle annular dark field (HAADF) mode using an Hitachi HD-2000, with an accelerating voltage of 200 kV. GOx CECs show an average area of $78 \mu\text{m}^2$, and contain a large concentration of protein as indicated by the high contrast in comparison to either the pre-CEC CNTs (a) or the CEC-containing CNTs of the nanocomposite (b).

Figure 4. Cyclic voltammetric characterization of CNT-Nafion (dotted) and CEC-CNT-Nafion (solid) electrodes. CEC-CNTs were generated from a 100% wt/wt GOx:CNT ratio, and CV measurements were taken in phosphate buffer at a scan rate of $100 \text{ mV} \cdot \text{s}^{-1}$. The cathodic and anodic peaks were observed at -0.462 V and -0.539 V , respectively ($E_p = 0.077 \text{ V}$), with an observed peak height ration (E_O) of $\sim 1 \pm 5\%$.

Figure 5.

Biofuel cell performance using a CEC-CNT nanocomposite based anode with glucose as fuel. BFC performance was collected at 25 °C from a MEA composed of a 100% GOx CEC-CNT with 200 mM glucose and oxygen as fuel and oxidant, respectively. BFC cell voltage (•) and power density (○) were monitored as a function of current density; the peak power density of CEC-CNT containing BFCs was observed to be $\sim 180 \mu\text{W}\cdot\text{cm}^{-2}$.

Table 1

BFC Outputs from Different GOx-Based Bioanodes

Bioanode	Scaffold	Immobilization ¹	DET/ MET	Mediator ²	Peak Power ($\mu\text{W}\cdot\text{cm}^{-2}$)	Reference
GOx	CNTs	CEC	DET	-	180	This Work
GOx	CNTs	CEC	DET	-	117	[30]
GOx	Graphene	CEC	DET	-	250	[29]
GOx/Catalase	CNTs	MC	DET	-	1250	[16]
GOx	CNTs	GAC	MET	DHB	45	[35]
GOx	CNTs	-	MET	TMPD	0.28	[21]
GOx	Graphite	MC	MET	PQ	40.8	[32]
GOx	Graphene	PP	MET	FC	78.3	[63]
GOx	Carbon fiber	PEG-HG	MET	PVP	280	[65]
GOx	Carbon fiber	PEG-HG	MET	PVP	350	[64]
GOx	Pt	AC-EPD	MET	BMIM	36	[37]

¹Immobilization Methods: AC-EPD, alternating current electrophoretic deposition; CEC, cross-linked enzyme clusters; GAC; gluteraldehyde crosslinking; MC, mechanical compression; PEG-HG, poly(ethylene glycol) (400) diglycidyl ether hydrogel; PP, polypyrrole

²MET Redox Mediators: BMIM, 1-butyl-3-methylimidazolium; DHB, 2,5-dihydroxybenzaldehyde; FM, ferrocene; TMPD, N,N,N,N -tetramethyl-phenylenediamine; PQ, phenanthrenequinone; PVP, poly(N-vinyl-2-pyridine)-[Os(N,N -dialkylated-2,2 -bi-imidazole)₃]²⁺³⁺; UB, ubiquinone