Carbon 107 (2016) 542–547

Contents lists available at ScienceDirect

Carbon

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

# Robust bifunctional buckypapers from carbon nanotubes and polynorbornene copolymers for flexible engineering of enzymatic bioelectrodes

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ARTICLE INFO

*Article history:*
Received 12 April 2016
Received in revised form
10 June 2016
Accepted 11 June 2016
Available online 13 June 2016

ABSTRACT

Enzymatic biofuel cells offer the exciting prospect of clean energy production for implantable devices, but such devices are still exotic and require improvements in electrode design and performance. Here a global strategy to prepare robust and versatile buckypaper bioelectrodes for advancing biofuel cell applications is presented. The fabrication method is based on a combination of original bifunctional polynorbornene copolymers with carbon nanotubes. Use of copolymers containing both pyrene and activated ester groups for cross-linking and tethering, respectively, increases the mechanical and electrochemical performance compared to buckypaper prepared without polymer or with the pyrene homopolymer. The amount of polymer used is an important parameter and was optimized to improve mechanical performance. High surface concentrations of reactive ester functionalities were obtained using long-chain polymers and exhibited high selectivity for attachment of aminoanthraquinone and the enzyme laccase. High performance biocathodes for direct oxygen reduction were constructed by immobilization of laccase on unmodified and anthraquinone-modified buckypapers. Anthraquinone-modified electrodes gave increased current densities due to improved electrical wiring of laccase via the hydrophobic pocket near the laccase T1 site. Biocathode stability over one month was excellent (53% current density after 24 days) and thus a new class of practical carbon-based enzymatic biofuels is envisioned.



## 1. Introduction

Enzymatic biofuel cells (EBFCs) offer great promise as low-power electricity sources for wearable and implantable bioelectronic devices from medical devices to environmental biosensors [1–3]. Tremendous advances have been made towards implantable fuel cells since the late 2000's and in particular for glucose EBFCs [2] where operation in vivo has been demonstrated, for example, in the retroperitoneal space of a rat [4] and in the body of a snail [5]. However, major technical challenges remain for implantable biofuel cells, including limited operational stability and modest power output at physiological pH [6,7]. Key challenges in EBFC design are the development of electrodes which offer (i) stable immobilization of enzymes and mediators, (ii) electrical wiring with the active site of enzymes, (iii) efficient diffusional transport in the cell, and (iv) are lightweight and easily integrated into devices.

Carbon nanotubes (CNTs) have great advantages as an electrode material for bioelectrodes due to their large surface area and exceptional electronic and mechanical properties which allow enhanced direct and mediated electron transfer [8,9]. Carbon nanotube bioelectrodes can be prepared by drop casting [10], spinning [11], 3D printing [12] and direct growth [13] onto a conductive support, by mechanical compression into free-standing disks [8], or by filtration to give free-standing or supported thin films (buckypaper, BP) [14–16]. BP bioelectrodes are commonly

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http://dx.doi.org/10.1016/j.carbon.2016.06.027
0008-6223/

prepared on supporting substrates, such as Toray paper, to improve stability and prevent nanotube release into the local environment [17–19]. Free-standing buckypaper tends to be brittle and difficult to manipulate but is desired for device integration and to minimize contact resistance and mass transport limitations [5,17,20]. Free-standing BP has several advantages over other CNT materials: it is extremely thin, lightweight and flexible, no electrode support is required, and it is easily processed into different shapes and sizes.

Surface functionalization techniques are essential for the immobilization and stabilization of enzymes on electrodes for biofuel cell applications [7]. Among the few reports available, the best enzyme immobilization methods are adsorption [8], encapsulation via Nafion [21] and hydrogel [22], and via surface modification with pyrene derivatives [5,14,20]. For example, the recent work by Bourourou et al. demonstrated functionalization of free-standing BP via bis-pyrene-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) as a simple and stable platform for enzyme wiring [20]. The presence of two pyrene groups on the molecule offered reinforcement of the buckypaper via nanotube cross-linking. However, the close proximity of the two pyrene groups leads to a low probability of connecting two CNTs. A more effective approach to improve cross-linking of CNTs is to incorporate long linear polymer chains presenting at least ten pyrene groups, although use of polymers requires careful attention to avoid poorly conducting materials that provide inefficient electron transfer and diffusional barriers [15].

Here we report the optimization and preparation of a new class of reinforced free-standing buckypapers using multifunctional polynorbornenes. Use of the BP materials for coupling anthraquinone via amide bond formation is investigated and a biocathode for direct oxygen reduction via immobilised laccase from *Trametes versicolor* is demonstrated.

## 2. Experimental section

### 2.1. Materials and apparatus

Mono-sodium phosphate monohydrate (98–102%), di-sodium hydrogen phosphate heptahydrate (98–102%), sulfuric acid (95–98%), 2-aminoanthraquinone (technical grade), ethanol (EtOH, ≥ 99.8%), *N,N*-dimethylformamide (DMF, 99.9%) and laccase (13.6 U $mg^{-1}$) from *Trametes versicolor* were purchased from Sigma Aldrich and used as received. Dry solvents were used directly from a drying and degassing solvent tower delivery system. Aqueous solutions were prepared from ultrapure water at 25 °C (resistivity ≥ 18.2 MΩcm). Laccase was stored at 4 °C. Commercial grade multi-walled carbon nanotubes (MWCNTs, 9.5 nm diameter, > 95% purity) were obtained from Nanocyl and used as received without purification. High purity oxygen and argon were obtained from Messer. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker DPX-400 or DRX-500 spectrometer in $CDCl_3$ unless otherwise stated. Chemical shifts are given in ppm downfield from the internal standard tetramethylsilane. Size exclusion chromatography (SEC) measurements were conducted using a Varian 390-LC-Multi detector suite fitted with differential refractive index (DRI), and UV/Vis detectors. A guard column (Varian Polymer Laboratories PLGel 5 μm, 50 × 7.5 mm) and two mixed D columns (Varian Polymer Laboratories PLGel 5 μm, 300 × 7.5 mm) were used. The mobile phase was chloroform with 2% trimethylamine eluent at a flow rate of 1.0 mL $min^{-1}$. SEC data was analysed using Cirrus v3.3 with calibration curves produced using Varian Polymer laboratories Easi-Vials linear poly(styrene) standards (162 Da–240 kDa).

### 2.2. Synthesis of polynorbornene homopolymer, random copolymers and block copolymers

The polynorbornene homopolymer P($Py_{50}$) (**1**) was synthesized via ring opening metathesis polymerization (ROMP) of (1-pyrenyl) methyl *exo*-5-norbornene-2-carboxylate (PyNb) as previously reported [15]. The polynorbornene random copolymers P($Py_{13}$-*co*-$NHS_{13}$) (**2**) and P($Py_{66}$-*co*-$NHS_{66}$) (**3**) and block copolymers P($Py_{6.5}$-*b*-$NHS_{13}$-*b*-$Py_{6.5}$) (**4**) and P($Py_{33}$-*b*-$NHS_{66}$-*b*-$Py_{33}$) (**5**) were synthesized via ROMP from pyNb and *N*-(2,5-dioxopyrrolidin-1-yl octanoate)-*cis*-5-norbornene-*exo*-dicarboximide (NHSNb) as described in the Supporting Information.

### 2.3. Preparation of polynorbornene buckypapers

First, 150 mg of MWCNTs were added into 50 mL DMF and dispersed by sonication for 30 min. Next, 6 mg of polymer was added into 3 mL DMF and dissolved by sonication for 5 min. 10% polymer: 90% CNT solutions were prepared by mixing 1.81 mL of polymer solution and 10.9 mL of CNT solution. 20% polymer: 80% CNT solutions were prepared by mixing 4.09 mL of polymer solution and 10.9 mL of MWCNT solution. 50% polymer: 50% CNT solutions were prepared by mixing 16.35 mL of polymer solution and 10.9 mL of MWCNT solution. All solutions were mixed by sonication for 15 min. The mixed solutions were subsequently passed through a Millipore PTFE filter (JHWP, 0.45 μM, 46 mm diameter) under high vacuum and left for 2 h. The buckypaper coated filters were left to dry flat at room temperature in the presence of desiccant, and then cut to size using a scalpel. Buckypapers were prepared using 80% CNT: 20% polymer in wt% solution unless otherwise stated.

### 2.4. Electrochemistry

Electrochemical measurements were performed at room temperature using an Eco Chemie Autloab PGSTAT 100 potentiostat running GPES 4.9 software. A conventional three-electrode cell setup was used for all electrochemical experiments comprising a buckypaper working electrode, a AgAgCl (sat. KCl) reference electrode and a Pt wire counter electrode. A small copper tape contact was added to the back of the buckypaper for electrical connection via a crocodile clip. Inter-electrode spacing was ≤0.5 cm. The surface area of the buckypaper electrodes was 0.46 $cm^2$. Surface concentrations of electroactive groups were determined from Equation S(1) according to Faraday's law (Equation S(1)) and electron transfer rate constants were estimated from Equation S(2) (see Supporting Information).

### 2.5. Surface coupling reactions

For anthraquinone coupling experiments, buckypapers were fully immersed in 2 mM 2-aminoanthraquinone in EtOH with stirring. Buckypapers were subsequently rinsed with EtOH and acetone then sonicated in EtOH for 1 min. For laccase coupling experiments, 5 mg/mL laccase was prepared in 0.1 M phosphate buffer (PB) pH 5 and incubated on the buckypaper substrate (25 μL) for 1 h at 4 °C, then thoroughly rinsed with PB before use. Laccase-modified electrodes were stored in 0.1 M PB pH 5 before and after use.

### 2.6. Mechanical properties and surface characterization

Tensile modulus and strength were measured using a Shimadzu AGS-X with a cross-head speed of 5 μm/s and a 20 N load cell. Sample dimensions were width = 20 mm, length = 30 mm and a specified thickness in the range = 0.08–0.2 mm. Calibration length