

Supplementary Information

Title: Membraneless Glucose/O₂ microfluidic biofuel cells using covalently bound enzymes

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Material and methods

2.1 Chemicals

Polyoxyethylenebis(diglycidylether) (PEGDGE, MW~525 g/mol), sodium dodecyl sulfate, 2,2'azinobis(-3-ethylbenzothiazoline-sulfonic acid)diammonium salt (ABTS), ferrocenemethanol (FEMOL), *D*-glucose, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), glucose oxidase (GOx) from *Aspergillus niger* (210 U/mg), ethanol and acetone were purchased from Sigma-Aldrich. Ultrapure O₂ and N₂ were from Air Liquide and Laccase from *Trametes versicolor* from Fluka (21 U/mg). 3-aminopropyltriethoxysilane was purchased from ABCR and single-walled carbon nanotubes from Cheap Tubes Inc. AZ 92560 and SU-8 photoresists were purchased from Microchemicals GmbH and AZ 400 k developer from AZ Electronic Materials. Polydimethylsiloxane was purchased from Sylgard and curing agent from Dow Corning Corp. CoTA laccase (2.6 U/mg) was produced and purified as described in¹.

2.2 Electrochemical measurements

Electrochemical measurements were performed using an Autolab PGSTAT100 potentiostat. A platinum wire counter electrode and an Ag/AgCl 0.1M aqueous KCl reference electrode were purchased from IJ Cambria.

2.3 Modification of the SWCNTs electrodes

A COOH-SWCNT electrode was patterned on a glass slide using lift-off lithography, as described in²: the pattern of the electrode was fabricated using positive photoresist AZ 9260, which upon development leaves openings on the exposed area. These openings were then exposed to 3-aminopropyltriethoxysilane and subsequently filled with a solution of nanotubes. The patterned area was recovered after submerging the substrate in acetone which dissolves the photoresist, and hence carries away the dried nanotube film resting on top. The nanotube film which rests on the glass

substrate stays even after the acetone submersion, owing to strong hydrophobic interaction between the nanotubes, as well as between the nanotubes and the modified glass substrate.

A glass slide was washed with MQ water and ethanol and then dried with N₂. 2 mL of positive photoresist AZ 9260 was spread on the glass slide using a spin coater (WS-400-6NPP-LITE; Laurell Tech. Corp). The glass slide was heated at 100°C for 4 min and then kept in a water saturated atmosphere for 1 min. The glass slide was then exposed 5 min to UV through the electrode pattern mask using a mask aligner (MJB3 ; SUSS MicroTech). The glass slide was then immersed in 50 mL of a 1:4 (AZ 400 K developer:MQ water) solution for 3 min and dried with N₂. The immersion was repeated 1 min until the pattern was completely developed. The electrode pattern was covered for 5 min with a 1% 3-aminopropyltriethoxysilane (w/w) in MQ water solution. The operation was repeated two times and the glass slide was dried with N₂. A solution of 1 mg.mL⁻¹ COOH-SWCNT in MQ water containing 1% sodium dodecyl sulfate (w/w) was sonicated for 1 h (Bendelin Sonoplus HD200, 75% per cycle, MS72/D). The glass slide was heated at 100°C and 20 μL of the COOH-SWCNT solution was pipetted on the electrode pattern. The operation was repeated three times to obtain a homogeneous surface after evaporation. The glass slide was then immersed for 2 h in acetone with magnetic stirring to eliminate unbound SWCNT. The glass slide was washed with MQ water and dried with N₂.

Polydimethylsiloxane (PDMS) was pasted onto the electrode to define the active surface (0.5 mm x 1 mm) after activation of the surfaces using O₂ plasma (Plasma system FEMTO; Diener Electronic). Subsequently, 20 μL of phosphate buffer (50 mM; pH7.5) containing 30 mM of EDC and 90 mM of NHS was pipetted onto the COOH-SWCNTs surface and allowed to react for 1 h at room temperature. The surface was then blow-dried with N₂ and afterwards, 5 μL of phosphate buffer (50 mM; pH7.5) containing the enzyme (amounts specified in Fig. 2 and 3) was added and allowed to react for 15 h at room temperature.

An NH₂-SWCNT electrode was created using the same process. 8 μL of phosphate buffer (50 mM; pH7.5) containing PEGDGE:enzyme in 2:1 (w/w) ratio (amounts specified in Fig. 2 and 3) were pipetted onto the NH₂-SWCNTs surface and dried for reticulation during 15 h at room temperature. Both electrodes were thoroughly rinsed with phosphate buffer (50 mM; pH7.5) before use.

2.4 Microfluidic biofuel cells

Pairs of NH₂-SWCNT or COOH-SWCNT electrodes were patterned as described in 2.3 on a glass slide with a separation of 1 mm, and modified with enzymes as described above. Separately, the rectangular microchannels were fabricated in PDMS using a soft lithography process³. A 1:9 PDMS:curing agent mixture was poured over a silicon wafer (Siltronix) patterned in relief with SU-8 photoresist. The mixture was degassed and heated at 65°C for 4 h for crosslinking. The structured

PDMS layer was peeled off the mould and the inlet and the outlet holes were punched with a 0.75mm diameter biopsy punch. Microchannels were sealed by bounding the PDMS to the patterned glass slide by exposing both parts to oxygen plasma, aligning with a binocular and pressing both parts together. The device consisted of a Y-shaped channel with two inlets and one outlet (Fig. 1). The microchannels were 2 mm wide and 110 μm deep. The fuel stream was phosphate buffer (50 mM; pH7.0) containing 100 mM NaClO_4 , 1 mM FEMOL and 100 mM *D*-glucose. The oxidant stream was O_2 saturated acetate buffer (50 mM; pH4.5) containing 100 mM NaClO_4 and 2 mM ABTS.

References

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