From “Cyborg” Lobsters to a Pacemaker Powered by Implantable Biofuel Cells

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Electronic supplementary information (ESI)

Chemicals: PQQ-dependent glucose dehydrogenase (PQQ-GDH; E.C. 1.1.5.2, from microorganism – not specified by the company) was purchased from Toyobo Co., Japan, and used as supplied. Laccase (E.C.1.10.3.2, from Trametes versicolor) was obtained from Sigma-Aldrich and used in experiments after the purification procedure described elsewhere.¹ 1-pyrenebutanoic acid succinimidy ester (PBSE) was purchased from AnaSpec Inc. Serum from a human male (type AB), 3-(N-morpholino)propanesulfonic acid (MOPS-buffer) and other standard chemicals were purchased from Sigma-Aldrich and used as supplied without any further purification. Water used in all of the experiments was ultra pure (18.2 MΩ·cm) from a NANOpure Diamond (Barnstead) source.

Pacemaker: The Pacemaker (Affinity DR 5330L, St. Jude Medical) was an explanted device that had been removed and discarded following battery depletion (as per standard pacemaker replacement protocol).

Electrode preparation: Buckypaper composed of compressed multi-walled carbon nanotubes (Buckeye Composites; NanoTechLabs, Yadkinville, NC) was used as the electrode material.
Electrodes were washed with isopropyl alcohol with moderate shaking for 15 min at room temperature prior to their modification. The electrodes were incubated with PBSE, 10 mM, in ethanol with moderate shaking for 1 h at room temperature, subsequently rinsed with ethanol to remove excess of PBSE and then with MOPS-buffer (50 mM, pH 7.0) to remove ethanol. The biocatalytic anodes were prepared by immobilization of PQQ-GDH: the PBSE-functionalized electrodes were incubated for 1 hour in the solution of PQQ-GDH (2.4 mg·mL⁻¹) in MOPS-buffer (50 mM, pH 7.0) containing Na₂SO₄ (100 mM) and 1 mM CaCl₂ (1 mM). The biocatalytic cathodes were prepared by immobilization of laccase: the PBSE-functionalized electrodes were incubated for 1 hour in the solution of laccase (1.5 mg·mL⁻¹) in potassium phosphate buffer (10 mM, pH 7.0). The immobilization reactions proceeded at room temperature with moderate shaking. Then the enzyme-modified electrodes were stored (4 °C) in the same buffer until implanted in lobsters for in vivo use or transferred to a biofuel flow cell for in vitro measurements. Characterization of the enzyme-modified electrodes (cyclic voltammetry, enzyme content, etc.) was described in details elsewhere.²,³

**Lobster and in vivo measurement:** The live specimens of American lobster (*Homarus americanus*) used in this study were purchased in a supermarket “Price Chopper” (NY). Lobsters were placed on a layer of crushed ice during experiments and upper part of the animal – where the electrodes were implanted – was exposed to ambient temperature. Two lateral sagittal rectangular slits were excised from the exoskeleton of the lobster cephalothorax. These slits were approximately 2 mm wide by 1 cm in length and cut through to the dorsal sinus of the venous system. The electrodes were then placed into the hemolymph between exoskeleton and stomach. The voltage and current generated by the biofuel cell were measured by a multimeter (Meterman 37XR) with a variable resistance used as an external load. All measurements were carried out at ambient temperature (23±2°C) with animals placed on crushed ice.

**Biofuel flow cell measurements:** The flow cell with the dimensions shown in the sketch, Figure ESI-1, included the buckypaper electrodes modified with PQQ-GDH and laccase in the anode and cathode, respectively, and the inlet/outlet of the cell were connected to plastic tubes of 0.5 mm internal diameter. Human serum solutions with the different glucose concentrations (3.7, 6.4
and 10.3 mM) were pumped (MINIPULS 3, Gilson) with the volumetric rates of 58.9 and 235.6 μL/min, mimicking blood flow in a human capillary of 0.008 mm with the liner rates of 1 and 4 mm/s characteristic of a resting or running person, respectively. Note that the volumetric flow rate was scaled up to generate the same liner rate in the tube of a bigger diameter. The polarization curves of the individual biofuel cell in the presence of different glucose concentrations and different flow rates were obtained by measuring voltage/current values on the external load resistance by a multimeter (Meterman 37XR). For assembling a power source with the $V_{oc}$ up to 3 V, five individual biofuel flow cells were connected to the pump by different tubes operating in parallel, while the electrodes were connected in series. The metal shell of the pacemaker (Affinity DR 5330L, St. Jude Medical) was cut and the device was opened, allowing to remove the internal battery and to connect the leads of the electronic chip to the external biofuel cell. The electrical output cable of the pacemaker was connected to an oscilloscope (Agilent DSO-3000) for recording the generated electrical pulses.

References


![Figure ESI-1. Sketch of the biofuel flow cell.](image-url)
Photos illustrating the experiments

Lobster prepared for the experiments
Lobster with the implanted biocatalytic electrodes
Measuring output voltage produced by the lobster's biofuel cell
Two lobsters with the implanted biofuel cells connected in series and used for powering the digital watch
The open pacemaker prior to its wiring to the external power source
The setup composed of five flow-biofuel cells used for powering the pacemaker (three biofuel cells are well visible in the front row, while two other biofuel cells are only partially visible in the back row)
Oscilloscope measuring the electrical pulses produced by the pacemaker activated by the biofuel cells