Wireless Communication by an Autonomous Self-Powered Cyborg Insect

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A trehalose/oxygen biofuel cell was implanted in Blaberus discoidalis to convert chemical energy stored within the insect hemolymph into electrical energy which was then used to power a custom-designed oscillator mounted on the back of the insect, capable of producing signals in the audible range. The ability of this cyborg to generate and transmit signals wirelessly was demonstrated by placing an external receiver up to a few centimeters away from the insect while it was tethered to a device that enabled it to walk in place on top of a light weight, air-suspended solid sphere. Wireless communication could also be established between the transmitter powered by the same type of biofuel cell implanted in the moth Manduca sexta and the receiver, while the live insect was being restrained with wax in a Petri dish. Possible means of reducing the weight and size of the transmitter so as to allow the moth to carry it in flight are discussed.

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Biofuel cells1,2 have emerged as promising devices for converting chemical energy into electrical energy in living organisms with potential application in a variety of areas of fundamental and technical relevance.3–6 Efforts in our laboratories have focused on the development of biofuel cells that could be implanted into insects6 and provide the power required not only for the operation of electronics for sensing, information storage and wireless communication, but also for the stimulation of the nervous system, a strategy that will ultimately allow control of certain aspects of the insect behavior. An attractive feature of this approach is that it provides a continuous and autonomous source of power thereby avoiding the need for an external battery as implemented recently by Bozkurt et al.,7 who succeeded

Experimental

Electrochemistry.— Enzyme-modified electrodes were prepared by depositing either a mixture of glucose oxidase (Aspergillus niger; EC 1.1.3.4, 153 units mg−1), trehalase (0.25 mg/mL expressed and purified in our laboratory), PVI-(dmnpbpy)2Cl3 (10 mg/mL), and PEGDGE (2.5 mg/mL) (Polysciences, Inc.) where PVI denotes poly(1-vinylimidazole), and dmnpbpy dimethyl bipyrindine and PEGDGE poly(ethylene glycol) (400) diglycidyl ether for the anode or of bilirubin oxidase (Trachyderma tsunodae, Amano, EC 1.3.3.5, 2.1 units mg−1), PVI-bpy2Cl2 (10 mg/mL) and PEGDGE for the cathode, as described previously,8 onto the flat end surface of individual graphite rods (99.9%, 2 mm thick, Graphite Store, for the anode) or carbon fiber bundles (1 mm thick, Hobby Lobby, for the cathode), ca. 2 cm in length. A gold wire was used to connect the electrodes to the electronics. A glass tube of small diameter ending in a ceramic frit filled with commercial phosphate buffer saline (PBS) solution (Fischer BioReagents of composition, 1.37 M sodium chloride, 0.027 M potassium chloride and 0.199 M phosphate buffer) after a tenfold dilution, was used as the cathode compartment. All enzyme-modified electrodes were tested in trehalose solutions just prior to implantation to validate their good bioelectrochemical performance.

Power curves were recorded by monitoring the steady-state current following application of five different potentials, i.e. 0, 0.1, 0.2, 0.3 and 0.4 V, using one of three different potentiostats, i.e. Autolab PGSTAT302N, CH1700F Electrochemical Analyzer 50, and Pine Instrument Co., yielding virtually identical results.

Electronics.— The maximum output power provided by the type of biofuel cell employed in this work was found to be of ca. 0.12 μW at 100 mV, decreasing gradually down to very low values as the voltage approached 300 mV (see solid circles, in Panel B, Fig. 1). At these very low supply voltages, MOSFET transistors lose their excellent switching properties since a theoretically minimum gate-voltage change of 60 mV, more typically 70 to 80 mV, is required to modulate the drain current by ten times.9 Also, most commercially available MOSFETs require a gate voltage of at least 0.5 V, i.e. the threshold voltage, to achieve a useful level of operating current. These constraints created a significant challenge to the design of an electronic circuit that could be powered by the biofuel cell, while providing a wireless indication that the circuit was indeed operating. An oscillator was chosen to be the most useful demonstration circuit since it serves as a key building block in data acquisition and communications systems. More specifically, a three-stage RC oscillator using inverters formed from NMOS transistors with resistive loads and capacitive feedback from third to first stage (Panel A,
Fig. 1) was selected for its ability to operate effectively at low voltage and power levels, with imperfect MOSFET switches. Low-threshold MOSFETs (ALD110802 from Advanced Linear Devices) were used with resistive loads of 2 MΩ to obtain low-power oscillation, and the RC feedback was chosen for a nominal oscillation frequency of 4.2 kHz, an audible frequency that is useful for demonstration. The oscillator was shown to function at supply voltages as low as 0.17 V, but its load characteristic combined with the source characteristic of the biofuel cell yielded operation at about 0.24 V and 0.07 μW, as shown in Panel B, Fig. 1.

Photographs of the circuit board, Blaberus discoidalis and that of a quarter, for comparison, are displayed in Fig. 2.

In wireless tests, the oscillator supply current flowed through a resonant LC circuit that served as an antenna, emitting an alternating magnetic field to a sensitive receiver that was tuned to the nominal oscillation frequency. In bench tests, laboratory equipment could be used to detect the wireless signal at distances as long as 15 cm. A researcher with a simple ear-piece could hear the tone at a distance of about 5 cm. The oscillator was subsequently powered directly from the biofuel cell immersed in a trehalose solution or implanted in the insects with surprisingly little difficulty (vide infra).

Insects handling.—Adult female Blaberus discoidalis cockroaches (see A, Fig. 2) were maintained in a 26 ± 1°C room on a 12h/12h light/dark cycle and housed in 19 L plastic buckets with screened lids, with free access to water and a chicken-mash grain food. The insects were anesthetized using CO2. The wings were removed distal to their thoracic attachment points (this prevents bleeding) to allow for dorsal abdominal implantation of the biofuel cell electrodes. Small incisions were then made in the cuticle, outward ca. 0.5 cm on both sides of the midline, to allow for later insertion of the electrode into a small glass tube ending in a frit filled with PBS solution housing the enzymatic cathode. The circuit board was attached to a flexible plastic tether piece (1.0 × 7.0 cm) that was slightly kinked to make a shallow “V” shape. The board occupied the longer, posterior part of the tether (suspending it centrally over the abdomen) and the kink was attached to the pronotum of the animal with a small amount of hot melt glue. The smaller anterior extension of the tether was used to suspend the insect above a six inch diameter Styrofoam ball that rode on pressurized air in a semicircular cradle, similar to the apparatus described by Hedwig and Poulet. The cockroach could thus move all of its legs and walk in place. Once walking had been established, with the circuit board mounted on B. discoidalis and the leads soldered to the board, the electrode and the glass capillary were inserted into the incisions made in the abdomen of the insect (see Fig. 3).
The animal was induced to walk normally by a light tap to the body. Once the measurements were completed, the tether was removed from the suspension mount above the ball and the animal showed completely normal walking when released onto the floor surface, both with and without the circuit board still attached.

*Manduca sexta* moths were maintained on a 14:10 hour light:dark daily cycle at ca. 28°C and a relative humidity of ca. 65%. Prior to the experiments, individual moths were cold anesthetized by placing them in plastic containers (8 cm in diameter and 6 cm in depth) and then onto crushed ice for ca. 20 min. They then had most of the scales on their bodies removed using a stream of compressed air, and then onto crushed ice for ca. 20 min. They then had most of the scales on their bodies removed using a stream of compressed air, and were subsequently restrained in a Petri dish with strips of dental wax across the legs and wings as shown in Fig. 4. Additional restraint was provided by three custom-fitted metal staples made from insect pins fitted around the neck, anterior and posterior of the abdomen. Two small incisions were made in the soft lateral cuticle on either side of the abdomen. These incisions were made in different segments and used to insert the trehalose anode and the glass capillary (see above). Once the measurements had been completed, moths were released by removing the staples and restraining wax strips and then allowed to move freely to ensure the procedure did not harm them.

*Wireless communication testing.*— Prior to the in vivo tests the biofuel cell electrodes were immersed in a non-deaerated 30 mM trehalose solution and connected to the oscillator. A clearly audible signal could be heard by placing the receiver close to the oscillator, which became more intense as the separation between oscillator and receiver was decreased. As expected, no signal could be heard upon removing one of the electrodes from the solution (*vide infra*). The same protocol was employed for in vivo measurements involving both *cockroaches* and *moths* for which video-audio footage was recorded on a Sony DCR-HC21 MiniDV camera, and later played back allowing the frequency to be analyzed using Spectrum Lab V2.77b22 software.

**Results and Discussion**

Shown in Fig. 5 are plots of power density vs. current density for the trehalose-oxygen biofuel cell implanted in *Blaberus discoidalis* (blue solid circle) and in *M. sexta* (black solid circles) yielding maximum power density values in the range of about 15 and 9 μW/cm², respectively. Also displayed in this figure are data collected with the two enzymatic electrodes in 30 mM trehalose solutions (open blue circles) for which the maximum power densities were found to be of about 12 μW/cm².

The ability of the fuel cell implanted in a live and moving insect to power the oscillator mounted on its body was demonstrated by bringing the receiver near to a tethered cockroach walking on a suspended Styrofoam ball as shown in Fig. 3, yielding an audible signal for distances of less than about 2 cm from the insect. A movie showing the entire arrangement in action may be accessed through the following link: [http://jes.ecsdl.org/content/161/13/H3113/suppl/DC1](http://jes.ecsdl.org/content/161/13/H3113/suppl/DC1). More detailed tests were performed while the cockroach was restrained on the bench by methods specified elsewhere. Shown in Fig. 6 is the frequency of the sound generated as the anode was inserted and then removed from the abdomen of the insect based on the analysis of data.

![Image](image-url)
performed with the instrumentation and software specified in the Experimental Section. Specifically, as the anode was first (t = 0) inserted into the insect (labeled as A-in), the frequency of the sound emitted was ca. 4.15 kHz which continued for the next 30 s at which point the anode was removed (A-out) leading to a decrease in the frequency until no sound could be heard (●). The same protocol was followed twice more yielding the same results (see sequences labeled as B and C) providing unambiguous evidence that the signals generated were produced using the biofuel cell as the power source. The gradual fading in and out of the frequency upon insertion and extraction of the electrode was simply caused by the 10-μF capacitor at the input of the oscillator, which had to be charged by the biofuel cell upon insertion, and discharged by the oscillator upon extraction. The RC oscillator has a frequency that is slightly voltage dependent, decreasing as voltage decreases (see Supplementary Material), and failing when voltage drops below 0.17 V.

Concluding Remarks and Future Outlook

The results of this investigation have shown that judicious design of the electronic circuitry allows for a single biofuel cell implanted in either B. discoidalis cockroaches or M. sexta moths to power continuously an oscillator capable of transmitting signals wirelessly to an external receiver. This is in stark contrast with work published in the literature in which two or more living organisms, either lobsters13 or cockroaches,15 connected in series, were required to generate sufficient biofuel cell derived power to drive an electronic circuit. Although the weight and volume of the circuit board involved in our studies was found to be small enough to allow B. discoidalis to move freely, it was too heavy for the moth to carry it in flight. We estimate, however, that a telemetry pack based on a custom integrated circuit with power conditioning similar to13 and optimized packaging (flip-chip bonding, etc.) would be about 15 mm² by 1 mm thick, with a mass less than 0.1 gram. Biofuel cells are in many respects better suited for power intensive applications than, for example, other strategies such as the elegant work of Mercier et al.,14 where the power was generated by the endocochlear potential and stored over a period of time before utilization.

It is interesting to compare biofuel cells with other means of energy harvesting for living organisms. Unfortunately, this is not as straightforward as one may think, as power densities in biofuel cells are quoted in W per unit cross-sectional area of electrode, whereas other more physical modes report power densities per unit weight. In fact, a very useful table that includes thermoelectric, piezoelectric and solar type transducers was recently published by workers at University of Michigan15 who listed values spanning from fractions of a mW/g, for e.g. body heat, to kW/g for a 1 cm² solar cell. One could in principle convert the biofuel cell data to gravimetric type units by dividing the power by the weight of the electrode which would indeed give very attractive values, given the very light weight of the polymer-enzyme layer. In fact, it seems feasible to use the cuticle of certain insects as the electrode support which would avoid adding extra weight. Yet another factor that would improve the performance metrics would entail changes in the overall architecture of the enzymatic cathode involving structures that resemble air permeable electrodes16 for conventional fuel cells. Efforts in this direction are being pursued in several laboratories including ours. Perhaps the most challenging aspect of biofuel cells relates to the long-term stability of the enzymes themselves. A strategy that could prove promising is the search of enzymes of the type required in extremophiles.

Acknowledgments

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