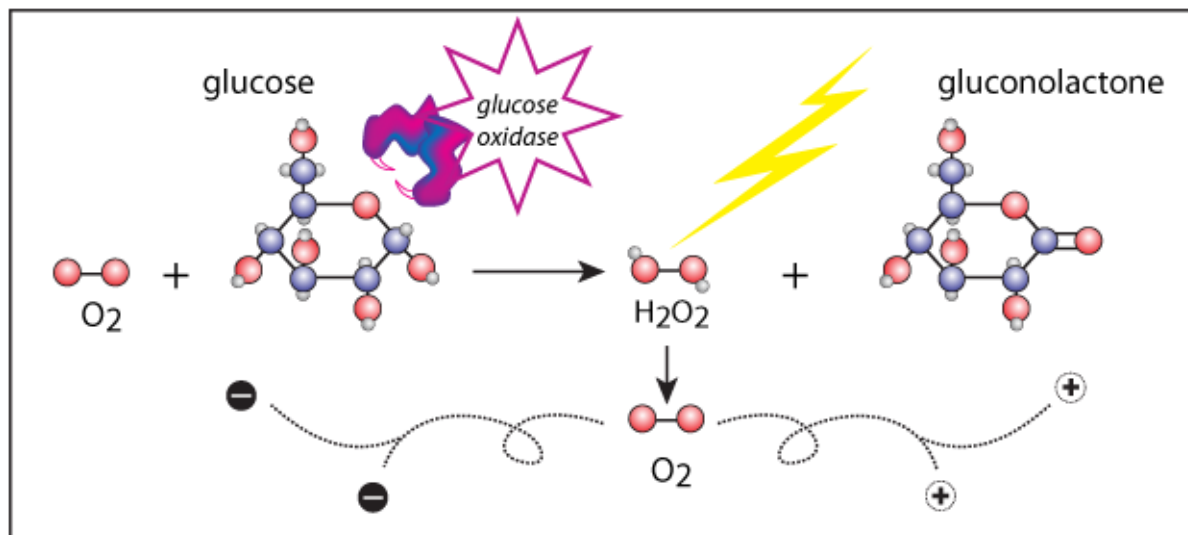


Bio Currents Research Center Protocol

Electrode Construction



Enzyme Assisted (Amperometric) Electrodes

The material below has been adapted from primary papers and several reviews originating from the BRC. These should be referred to in publications and for the original source materials.

References:

1. *Smith, P.J.S., Sanger, R.S. and Messerli, M.A. (2007) Principles, Development and Applications of Self-Referencing Electrochemical Microelectrodes to the Determination of Fluxes at Cell Membranes. In: Methods and New Frontiers in Neuroscience. Ed. Adrian C. Michael. CRC Press. Ch. 18: 373-405.*
2. *Messerli, M.A., Robinson, K.R. and Smith, P.J.S. (2006) Electrochemical sensor applications to the study of molecular physiology and analyte flux in plants. In: Plant Electrophysiology - Theory and Methods. Ed. Alexander G. Volkov. Springer-Verlag. 73-107.*

Platinum Based Microelectrodes

Building the Reactive Surface

Platinum wire, 25 μm in diameter, (1.5 cm length: Johnson Matthey, Ward Hill, MA) is straightened by rubbing with cardboard against a flat surface. These wires are then glued into 27 gauge hypodermic tubes (5 cm long: Small Parts, Inc., Miami Lakes, FL) with silver epoxy (Epoxy Technology, Inc., Billerica, MA) so that the Pt wire extends ~ 1 cm from the tip. The wire is then electrochemically etched in an aqueous solution of 3 M KCN and 1 M NaOH by applying square waves of 4.0 V amplitude and a 4 ms period (pulse generator model 114 Tektronix, Inc., Portland, OR) until the tips are reduced to a fine point of $< 1 \mu\text{m}$. The etched Pt wires are rinsed with water and isopropyl alcohol and inserted into borosilicate glass capillaries (1B150, WPI) pulled to outer tip diameters of $\sim 3 \mu\text{m}$ using a Sutter P-97, so that the wire protrudes slightly from the pipette tip. The electrodes are dipped into epoxy (Epon 828 with m-phenylenediamine curing agent; Miller-Stephenson, Danbury, CT) and heated to $90 \pm 6 \text{ }^\circ\text{C}$ on a hot plate. The electrodes dry overnight. At this stage the epoxy has sealed the Pt to the glass. A thin layer of epoxy probably coats the Pt protruding from the glass. Briefly dipping into acetone cleans the Pt. The epoxy is then cured by baking at $100 \text{ }^\circ\text{C}$ for 2 h and $150 \text{ }^\circ\text{C}$ for 2 h. The exposed Pt electrodes are etched again (same conditions as above) to form a recessed electrode with a cavity 2-3 μm deep. This etching takes no more than 5 min and can be monitored by examining the electrode under a microscope every 60 sec. Finally, the electrodes are coated by dipping in a solution of 10% cellulose acetate (30 kDa: w/v in tetrahydrofuran, THF) for 60 s and drying for 10 min. In a more recent paper THF is replaced by acetone. A machined pin (Allied Electronics: part no. 900-0510) is attached to the tubing and inserted into a BNC connector with its pin removed. The finished assembly is coupled to the headstage (Fig.1)

Completing the circuit

An Ag/AgCl reference electrode, connected via a 3 M KCl/5% agar bridge and placed in the bulk solution, completes the circuit.

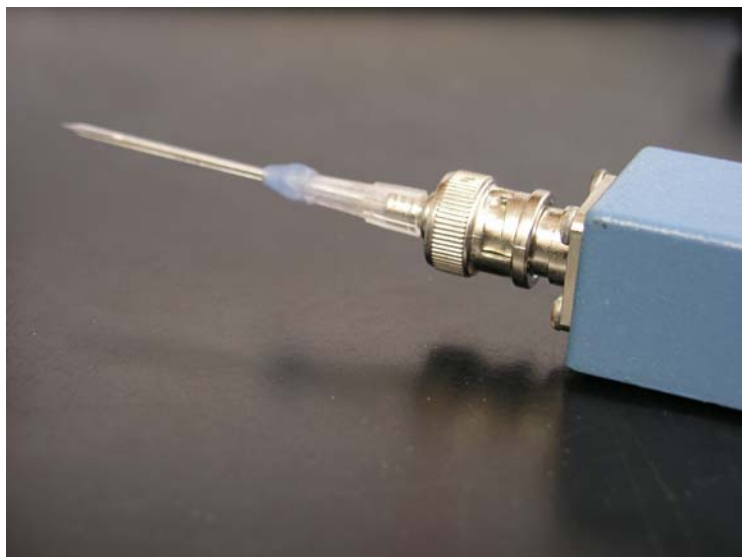


Figure 1: The final construction of the oxygen electrode attached to the BRC amperometric headstage via a modified BNC. A small piece of dental wax (blue) seals the glass to a trimmed pipette tip providing good stability.

1. Modification for a Glucose Electrode

Glucose microsensors are fabricated in the first instance as described above for oxygen electrodes. The exposed Pt wires are etched to form a cavity $\approx 5 \mu\text{m}$ deep. Once etched, Pt particles are electrochemically plated on the recessed Pt wire at -0.2 V vs Ag/AgCl in a solution of 10 mM hydrogen hexachloroplatinate (IV) hydrate (Cat # 20,608-3: Aldrich, Milwaukee, WI) for 3-5 min. The amount of charge required for platinization is $1.2 \pm 0.3 \times 10^{-5}$ coulombs (mean \pm SD, $n = 26$). Glucose oxidase (GOx: EC 1.1.3.4., type X-S from *Aspergillus niger*: Sigma, St. Louis, MO) is then loaded onto the platinum particles by immersing the tip of the electrodes in 1% (w/v) GOx solution for 10 min. The enzyme is immobilized with 10% glutaraldehyde vapor for 1 min. The immobilized enzyme layer is then coated with 10%, 30 kD, cellulose acetate (w/v in acetone) by dipping (10 sec) and drying (60 sec), repeated 4 times. The final tip diameter is $\sim 8 \mu\text{m}$.

Calibration of the glucose electrode, polarized to $+0.6 \text{ V}$, is against 2, 5 and 10mM glucose in 50mM/l HEPES and 150mM NaCl at pH 7.4 and 37°C . Experimentally the glucose electrode is operated at a frequency of 0.2 Hz over a $10 \mu\text{m}$ excursion. The speed of response to 90% is 0.46s.

2. Modification for a Glutamate Electrode⁵

Microelectrodes are fabricated in a manner similar to oxygen sensors, except that 8- μm carbon fiber, 12- μm gold wire, and 10- and 25- μm platinum wire are tested as a reactive surface. Electrodes are dip-coated with Os-gel-HRP redox polymer (BAS, W. Lafayette, IN) and allowed to dry for 10 min. Electrodes are then dip-coated in 50 units/ml glutamate oxidase from *Streptomyces sp.* (Cat. # G-0400, Sigma) and allowed to dry for 10–30 min. Glutamate electrodes are polarized to either 0 or $+100 \text{ mV}$ against a Ag/AgCl reference electrode, connected via a 3 M/l KCl/5% agar bridge placed in the bulk solution, to complete the circuit. The electrode is operated at a frequency of 0.1 Hz over a translational distance of $25 \mu\text{m}$. The average response of platinum-based electrodes was $0.45 \text{ pA}/\mu\text{M}$ of glutamate, whereas gold-based electrodes produce a weaker signal of $0.21 \text{ pA}/\mu\text{M}$, and carbon gave the weakest signal, $0.1 \text{ pA}/\mu\text{M}$. This electrode has not yet been used on a biological preparation.

3. Coming Soon: Lactate Electrode

4. Coming Soon: Glycerol Electrode

5. Coming Soon: Phosphate Electrode

***See Equipment and Software Protocol for equipment requirements**

Literature Cited:

3. Osbourn, D., Sanger, R.H. and Smith P.J.S. (2005) Determination of single cell oxygen consumption with impedance feedback for control of sample-probe separation. *Anal. Chem.* 77, 6999-7004.
4. Jung, S.-K., Trimarchi, J.R., Sanger, R.H. and Smith, P.J.S. (2001) Development and application of a self-referencing glucose microsensor for the measurement of glucose consumption by pancreatic b-cells. *Anal. Chem.*, 73, 3759-3767.
5. Bogorff, D.J., Messerli, M.A., Malchow, R.P. and Smith P.J. (2003) Development and characterization of a self-referencing glutamate-selective micro biosensor, *Biol. Bull.*, 205, 207-208.