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A Multifunctional Neural Microprobe for Simultaneous Multi-Analyte Sensing and Chemical Delivery

Bo Wang[1, 5†], Ximiao Wen[2†], Yan Cao[3], Shan Huang[4], Hoa Lam[1], Tingyi "Leo" Liu[6], Pei-Shan Chung[7], Harold G. Monbouquette[3], Pei-Yu Chiou[2,7]*, and Nigel T. Maidment[1]*

[1]Department of Psychiatry & Biobehavioral Sciences, Semel Institute for Neuroscience and Human Behavior, University of California at Los Angeles, California, USA

[2]Department of Mechanical and Aerospace Engineering, University of California at Los Angeles, Los Angeles, California, USA

[3]Department of Chemical and Biomolecular Engineering, University of California at Los Angeles, Los Angeles, California, USA

[4]Department of Biological Chemistry, University of California at Los Angeles, California, USA

[5]Department of Electrical and Computer Engineering, University of California at Los Angeles, California, USA

[6]Department of Mechanical and Industrial Engineering, University of Massachusetts Amherst, Massachusetts, USA

[7]Department of Bioengineering, University of California at Los Angeles, CA, USA

[†]These authors contributed equally to this work

*Corresponding authors at: University of California, Los Angeles, CA 90095, USA

Email Addresses: Nigel T. Maidment (nmaidmen@g.ucla.edu), Pei-Yu Chiou (pychiou@seas.ucla.edu)

Supplementary Methods

Reagents and Materials

Glucose oxidase (*Aspergillus niger*, 9001-37-0), choline oxidase (*Alcaligenes sp.*, 9028-67-5), m-phenylenediamine (PD), choline chloride, glutaraldehyde solution (25%), glutamate, bovine serum albumin (BSA) lyophilized powder, hydrogen peroxide solution (30%), 1H,1H,2H,2H-perfluorooctyltriethoxysilane, D-(+)-glucose, L-ascorbic acid, and dopamine hydrochloride were purchased from Sigma-Aldrich (St.Louis, MO). Glutamate oxidase (G4001-01, EC 1.4.3.11) was purchased from US Biological Life Sciences. Isopropyl alcohol and 1 M sulfuric acid solutions were obtained from Fisher Scientific (Pittsburgh, PA). Sodium phosphate buffer (PBS, pH 7.4) was composed of 50 mM sodium phosphate (dibasic) and 100 mM sodium chloride. Ultrapure water was generated using a Millipore Milli-Q Water System and was used for preparation of all solutions. Ag/AgCl glass-bodied reference electrodes with 3 M NaCl electrolyte and 0.5 mm-diameter Pt wire auxiliary electrodes were purchased from BASi (West Lafayette, IN). SU-8 2075 and SU-8 developer were obtained from MicroChem (Westborough, MA). The Sylgard® 184 silicone elastomer kit was purchased from Dow Corning (Auburn, MI). Four-inch Si wafers were purchased from Silicon Valley Microelectronics (Santa Clara, CA). The microelectrodes used in this work were silicon-based

multielectrode arrays (MEAs) produced in-house using microelectro-mechanical-system (MEMS) technologies.

Design and fabrication of the microprobe

Details of the design and fabrication of the silicon probes were reported previously[1]. Briefly, probes were fabricated in the following four major steps: i) A 1 µm thick layer of SiO_2 was thermally grown on a 150 µm-thick silicon wafer, ii) 20/100 nm-thick chromium/platinum was deposited and patterned to form electrodes and wires, iii) A 1 µm-thick SiO_2 passivation layer was deposited and patterned, and, iv) probes were released by deep reactive ion etching. The microelectrode array probes were 150 µm thick, 144 µm width, and 9 mm long. Each probe consists of four 6000 μm^2 (40 µm × 150 µm) platinum microelectrodes arranged in 2 pairs at the tip of the probe shank (0.144 × 9 mm, width × length) separated by 100 µm, and the sites within each pair are 40 µm apart.

A PDMS microfluidic channel with a cross-sectional dimension of 20 × 10 µm (width × height) was transferred to the back side of a silicon probe (Figure. 2a i-iii). The thickness of the microfluidic channel was 15 µm, which gave the final thickness of probe 165 µm; the width of the probe was not affected. Key steps of our previously described[2] PDMS thin-film transfer process are summarized as follows (Figure 2b):

i) silicon molds with structures at two different depths (5 and 15 µm) were created to determine the channel height and film thickness. The narrow 15 µm tall ridges serve as a "cutting-edge" to maximize the pressure between the silicon mold and the glass plate to eliminate PDMS film residues.

ii) PDMS was poured into the silicon mold and pressed by a glass slide to cure at room temperature. The PDMS was prepared by first mixing 10 g pre-polymer with 10 µL Pt catalyst (2% Pt in xylene; Gelest, Inc) and then mixing with 1 g cross-linker, which can be cured at room temperature within 6 hours. The silicon mold (3 µL, 6 hours deposition) and the glass slide (20 µL, 24 hours deposition) were previously coated with 98% 1H,1H,2H,2H-perfluorooctyltriethoxysilane (silane) *via* vacuum deposition to reduce stiction to PDMS. The stiction force can be tuned by changing the amount of silane in the vacuum chamber and deposition time.

iii) The glass slide was peeled-off, leaving the cured PDMS thin-film inside the mold due to the lower stiction of PDMS to the heavily silane-treated glass compared to the mildly silane-treated silicon mold.

iv) After a brief silane treatment of the thin-film (1 µL, 10 mins deposition), a second layer of PDMS was poured on top as a buffer, which was then covered by another silane-treated glass slide. The second PDMS buffer layer was ~5 mm thick, determined by a spacer placed between the silicon mold and the glass slide.

v) After curing, the PDMS thin-film was demolded, together with the PDMS buffer and the glass slide. The structures on the thin-film were dimensionally stable thanks to the rigid glass backing, enabling a highly precise alignment across a large area. The PDMS thin-film was bonded to the silicon probe after oxygen plasma activation (500 mT, 80 W, 30 s; Technics Micro-RIE, Series 800), which was temporarily fixed on a cover glass with photoresist (AZ 5214/non-exposed SU-8).

vi) The glass slide and PDMS buffer were peeled-off consecutively and the probe was released in acetone.

Calibration of flow rate

The performance of the microfluidic channel was tested in a brain phantom (0.6% agarose gel[3]) using an aqueous solution of Allura Red AC for visualization. The microprobe was connected to pre-loaded microfluidic tubing (BTPE-50, Instech Laboratories, Inc), which was in turn connected to a constant pressure source (FemtoJet, Eppendorf). The probe was then implanted into a brain phantom and flow rate was determined by the ratio between the measured change from preloaded volume and pumping duration at various pumping pressures (5-20 psi), averaged across 3 measures to assess reproducibility.

Biosensor Fabrication

The platinum microelectrodes on the microprobes were rinsed with isopropyl alcohol followed by an electrochemical cleaning step (-0.2 V – 1.5 V, 50 mV/s, 7 cycles, 0.5 M sulfuric acid) and sonication in DI water. The on-probe reference electrode was fabricated by electrodeposition of platinum nanoparticles (PtNPs) and IrOx. The PtNPs were deposited in a chemical reduction reaction using an aqueous solution of 2.5 mM H_2PtCl_6 and 1.5 mM formic acid (-0.1 V *vs.* Ag/AgCl, 10 min)[4-6]. Then, IrOx was electrodeposited following our previously described method[2, 5, 9]. The impedance of the IrOx electrode was measured at 1 kHz with HP Precision LCR Meter (4284A). A poly-m-phenylenediamine (PPD) film was electrodeposited on the remaining three Pt microelectrodes (5 mM PD in stirred phosphate-buffered saline (PBS), 0.85 V *vs.* Ag/AgCl, 10 min) followed by dip-coating of a Nafion layer (1% Nafion, 110 °C for 20 min).

For glucose sensor preparation, the microelectrode pair nearest the shank tip was manually coated with 12 layers of enzyme mixture consisting of glucose oxidase (8 mg/mL), BSA (6mg/mL), and glutaraldehyde (GAH, 0.075%). For glutamate and choline dual sensing, a modification of our previously described[7, 8] PDMS microstamping technique was employed (Figure. S1). Briefly, PDMS microstamps were fabricated using the Sylgard® 184 silicone elastomer kit. The PDMS microstamps were cured and detached from SU-8 mold and cut into 1 cm × 1 cm pieces (Figure S2). The size of a microstamp surface (50 µm × 160

μm) was designed to be slightly bigger than the size of the microelectrode. Glutamate oxidase (GlutOx, 0.25 unit/μl) or choline oxidase (ChOx, 0.5 unit/μl) was mixed with bovine serum albumin (BSA, 10 mg/ml) in PBS in a 1:1 (v/v) ratio. After ~20 min incubation with a microstamp, the GlutOx/BSA or ChOx/BSA mixtures were sequentially stamped (Figure S4) onto the upper right and bottom left sites of the microelectrode array, where the probe tip was supported by a PDMS structure shown in Figure S3, followed by 1 min exposure to 5% glutaraldehyde (GAH) solution for crosslinking. The remaining microelectrode was left as control site (upper right for glucose sensor; bottom right for glutamate/choline dual sensor). The PDMS stamps were cleaned in 7.5% hydrogen peroxide with sonication before re-use[7, 8].

Biosensor characterization in solution

Sensor development, evaluation and calibration in solution were performed using a Versatile Multichannel Potentiostat (model VMP3) equipped with the 'p' low current option and N'Stat box driven by EC-LAB software (Bio-Logic USA, LLC, Knoxville, TN) in a three-electrode configuration inside a Faraday cage. The PtNPs/IrOx electrode worked as reference/counter electrodes. To characterize the biosensor, aliquots of analytes (*i.e.* glucose, glutamate or choline) from stock solutions were added to a stirred solution of PBS (10 ml) to acquire the desired concentrations, while applying a potential of 0.6 V to the biosensor electrodes *vs.* IrOx[2, 5]. Additionally, aliquots of the potential interferents AA (250 μM final concentration) and DA (5-10 μM final concentration) were added to the beaker to determine the selectivity. Limit of Detection was calculated as 3 times the standard deviation of the baseline current (signal-to-noise ratio of 3, with no target added) divided by the slope of the sensor calibration curve[6].

Biosensor characterization in brain phantoms

Electrochemical sensing experiments in brain phantoms (0.6% agarose gel in artificial cerebrospinal fluid, aCSF: 125 mM NaCl, 2.5 mM KCl, 0.9 mM NaH_2PO_4, 5 mM Na_2HPO_4, 1.2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) were conducted with the same configuration as above. Microprobes were connected *via* pre-loaded microfluidic tubing (800 μM glucose; 800 μM glutamate and/or choline in aCSF), to a constant pressure source (FemtoJet, Eppendorf) and then implanted into a brain phantom to simulate the brain environment[3]. Amperometric data were collected and averaged over 0.1 s intervals and further processed using a moving average filter with 11 input points. Responses were recorded across a range of signal amplitudes by increasing the duration of pressure injection and thereby increasing the volume injected.

Acute *in vivo* studies

Male Sprague Dawley rats (300-628 g) were anesthetized with isoflurane and placed in a standard stereotaxic frame for surgery. All experimental procedures and surgeries were conducted in accordance with the Institutional Animal Care and Use Committee of UCLA. A microprobe, pre-calibrated in solution, was unilaterally implanted into the right striatum (A/P +1.0 from bregma, M/L +2.5 and D/V -5.0) according to the atlas of Paxinos and Watson (4th ed.). In addition, input tubing was closed, instead connecting to the atmosphere, when not injecting liquid to avoid blood backflow and clogging. Animals remained under anesthesia throughout the experiment. *In vivo* electrochemical sensing experiments were conducted with a multichannel FAST-16 potentiostat (Quanteon, LLC, Lexington, KY, USA) using a two-electrode system (0.6 V *vs.* IrOx) inside a Faraday cage. After the electrode signal reached equilibrium state (approximately 60 min), glucose (8 mM in aCSF) or glutamate/choline mixture (50 mM in aCSF) was pressure-injected (FemtoJet, Eppendorf) through the microfluidic channel. Amperometric data were collected at 80 kHz, averaged over 0.1 s intervals, and further processed using a moving average filter with 11 input points. Microprobes were post-calibrated in solution following removal from the brain.

Supplementary Figures

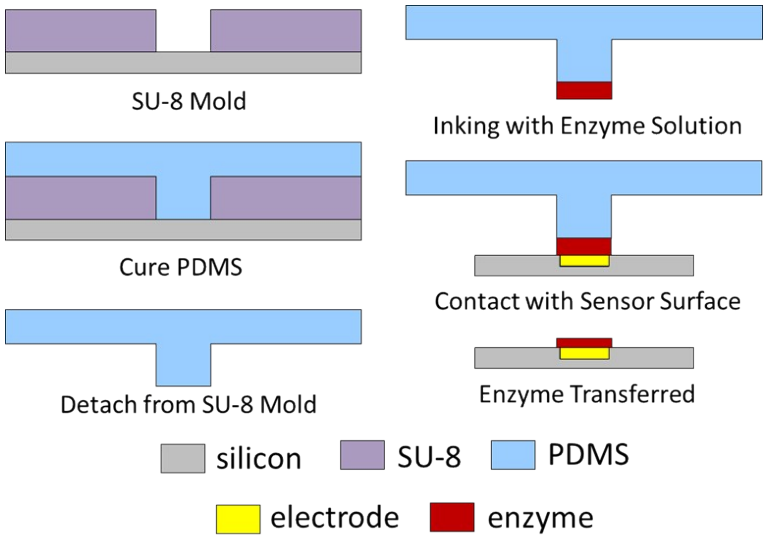

Fig. S1. Schematics showing the process of PDMS microstamp fabrication and enzyme stamping.

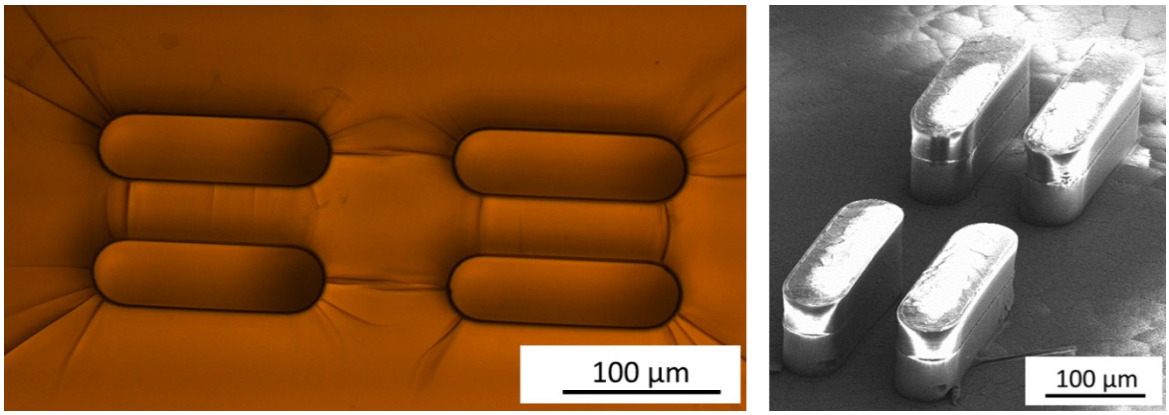

Fig. S2. Representative pictures of a SU-8 mold (2 × 2) and the corresponding cured stamp.

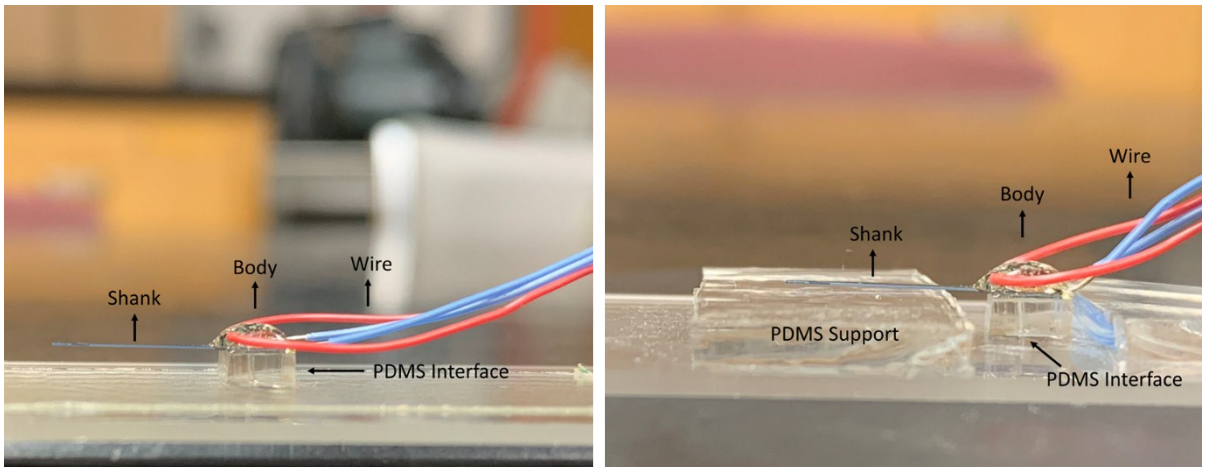

Fig. S3. Optical image showing the probe preparation before stamping (with PDMS support underneath the shank of the probe).

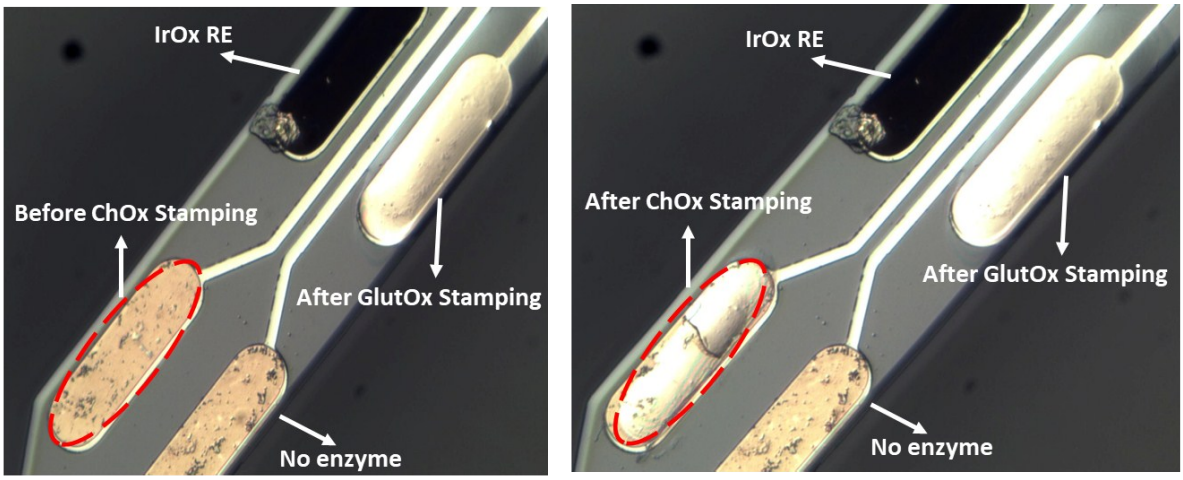

Fig. S4. Optical images showing the enzyme stamped (here, choline oxidase, ChOx) on the lower-left electrode of microelectrode array (Pre-functionalized with Pt nanoparticle-based IrOx reference electrode, IrOxRE, and stamped glutamate oxidase, GlutOx).

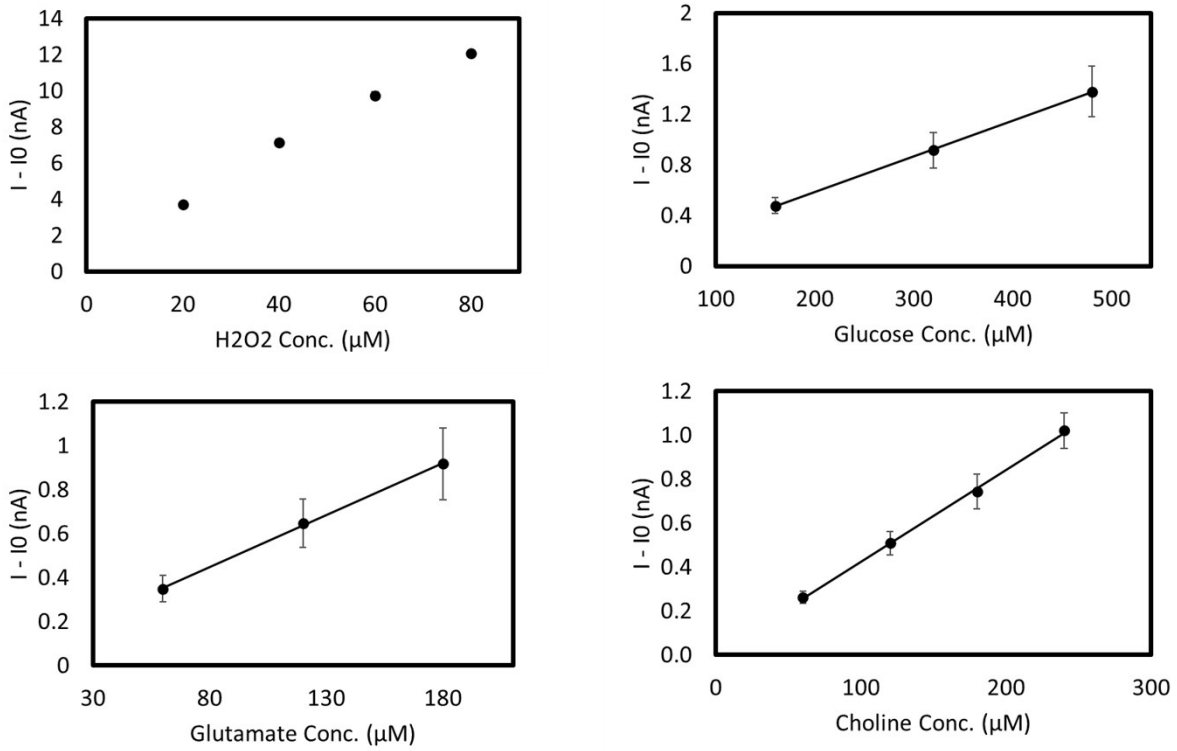

Fig. S5. Calibration curves for H_2O_2 (0 – 80 µM, n = 3), glucose (0 – 480 µM, n = 6), glutamate (0 – 180 µM, n = 3), and choline (0 – 240 uM, n = 4). Error bar: Standard error mean.

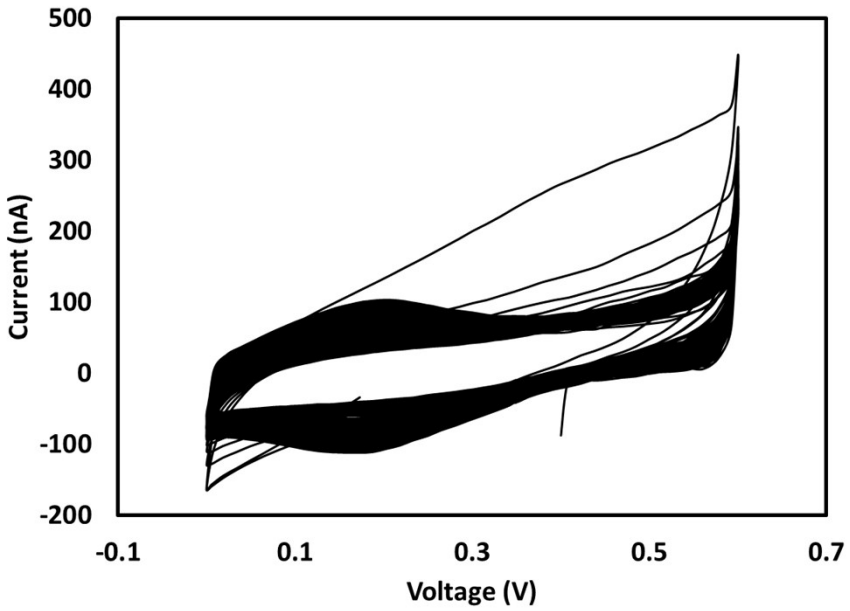

Fig. S6. A typical cyclic voltammogram curve for IrOx electrodeposition. 0.0 - 0.6 V, 100 cycles, 50 mV/s.

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