Supplemental Material

Simple fabrication of flexible biosensor arrays using direct writing for multi-analyte measurement from human astrocytes

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Biosensor Array Fabrication

The video included in supplemental material comprises three clips demonstrating the automated microfluidic dispensing system for direct writing. The first clip shows printing PtNP nanocomposite ink as conductive lines on a glass slide. The second clip shows printing the same ink as contact pads, conductive lines and electrodes. The final clip shows the dispensing system with a pulled-pipette needle printing a solution of enzyme matrix onto a microfabricated electrode array with 30-µm diameter electrodes.

Cyclic Voltammetry

We used cyclic voltammetry to characterize the effect of a Nafion® membrane and GFDMEM on biosensor electrodes and to select a potential for amperometry. During cyclic voltammetry, current is a function of capacitive charge accumulation between electrode and electrolyte (e.g., PBS) (non-Faradaic current) and a function of analyte gradient at the electrode[1]. Electrochemical reactions (Faradaic current) and mass transfer determine the analyte gradient. We measured all cyclic voltammograms with a three-electrode setup and from 0.8 V to -0.6 V vs. Ag/AgCl at a scan rate of 10 mV/s for 3 cycles. The reference electrode was Ag/AgCl in 3 M NaCl, and the counter electrode was a graphite rod.

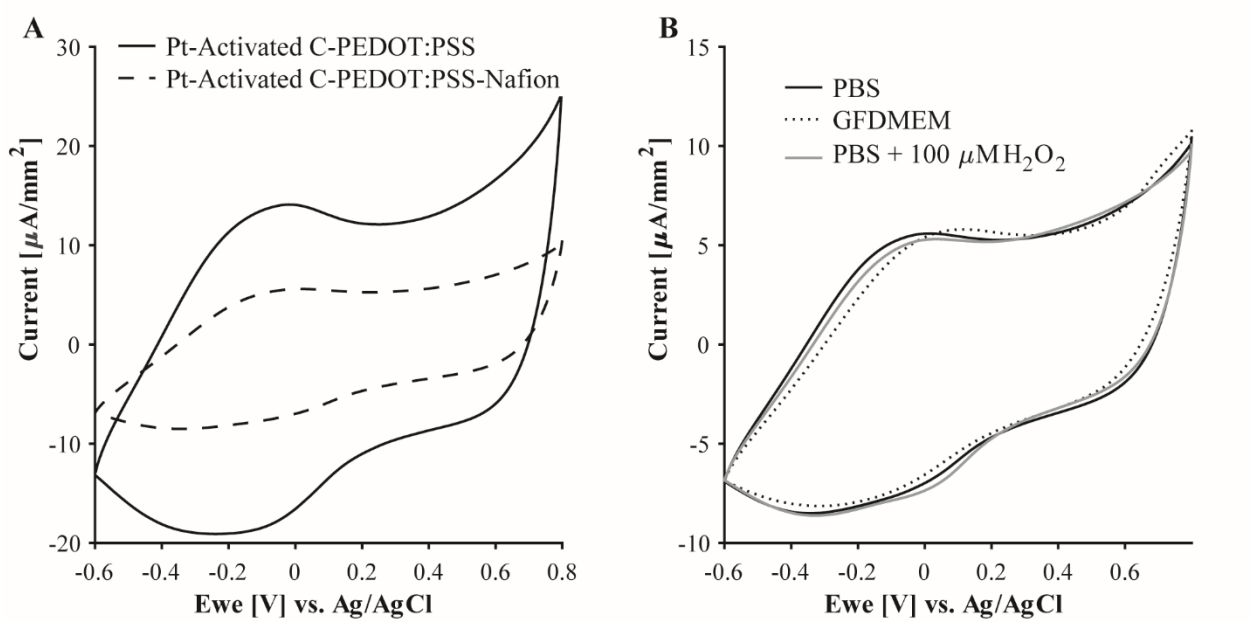

Figure S1. Cyclic voltammetry. (**A**) Pt-activated C-PEDOT:PSS composite electrodes with and without Nafion in 0.01 M PBS (pH 7.4). (**B**) Pt-activated C-PEDOT:PSS composite electrodes with Nafion in 0.01 M PBS, glucose-free DMEM (GFDMEM) and 0.01 M PBS + 100 µM H_2O_2.

The Nafion membrane sits between the biosensor electrode and enzyme layer. Nafion allows H_2O_2 from lactate, glutamate and glucose oxidation to pass to the electrode while excluding anions, such as ascorbic acid, that may interfere with the biosensors. **Fig. 1A** shows that current as a function of potential decreases from Pt-Activated C-PEDOT:PSS electrodes to the same electrodes with a Nafion membrane (Pt-Activated C-PEDOT:PSS-Nafion) but not entirely. Therefore, the biosensor electrodes remain conductive after adding a Nafion membrane although Nafion reduces their conductivity.

The cyclic voltammogram of Pt-Activated C-PEDOT:PSS-Nafion in glucose-free DMEM (GFDMEM) in **Fig. 1B,** compared to the same electrode in PBS, lacks any current peak until beyond 0.6 vs. Ag/AgCl. This suggests minimal interfering reactions from GFDMEM ingredients when biasing biosensors at 0.5 V vs. Ag/AgCl. On the other hand, **Fig. 1B** shows that adding 100 µM H_2O_2 increases current at 0.5 V vs. Ag/AgCl.

Multi-Analyte Measurement from Human Astrocytes

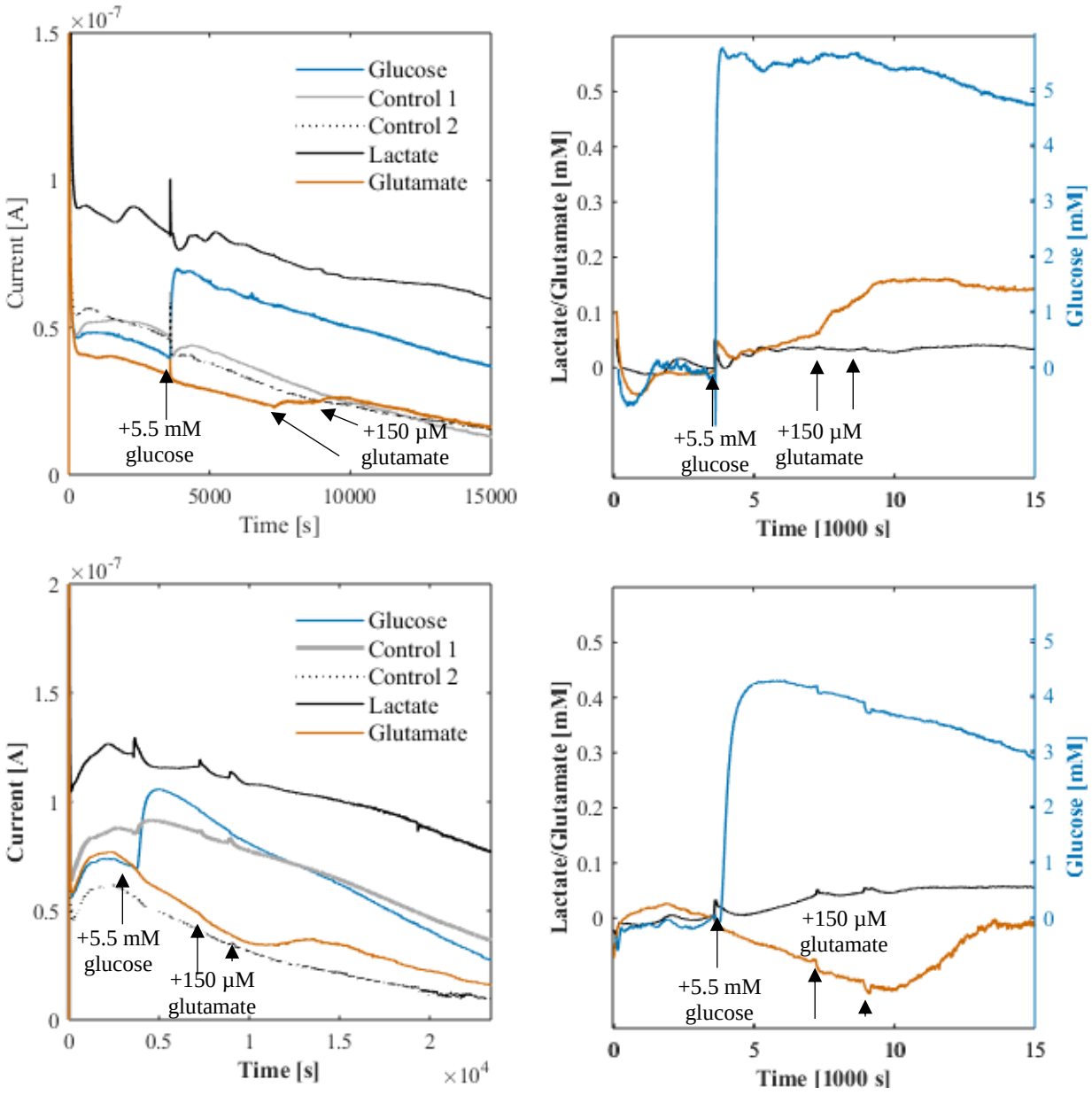

Figure S2. (**A, C**) Raw data of current as a function of time from two other measurements from 1 mm above human astrocytes in GFDMEM in a humidified incubator at 37°C, 5% CO_2. We added 5.5 mM glucose at 1 h, 0.15 mM glutamate at 2 h and another 0.15 mM glutamate at 2.5 h. (**B, D**) Lactate, glucose and glutamate as a function of time calculated from data in (**A, C**), respectively. Note that the glucose response is significantly larger due to larger glucose dosage to reflect physiologically relevant concentration. Also, glutamate response was delayed in (**C, D**). We suspect this was due to slower mixing on account of adding boluses of higher concentration (40 mM vs. 4 mM), but smaller volume.

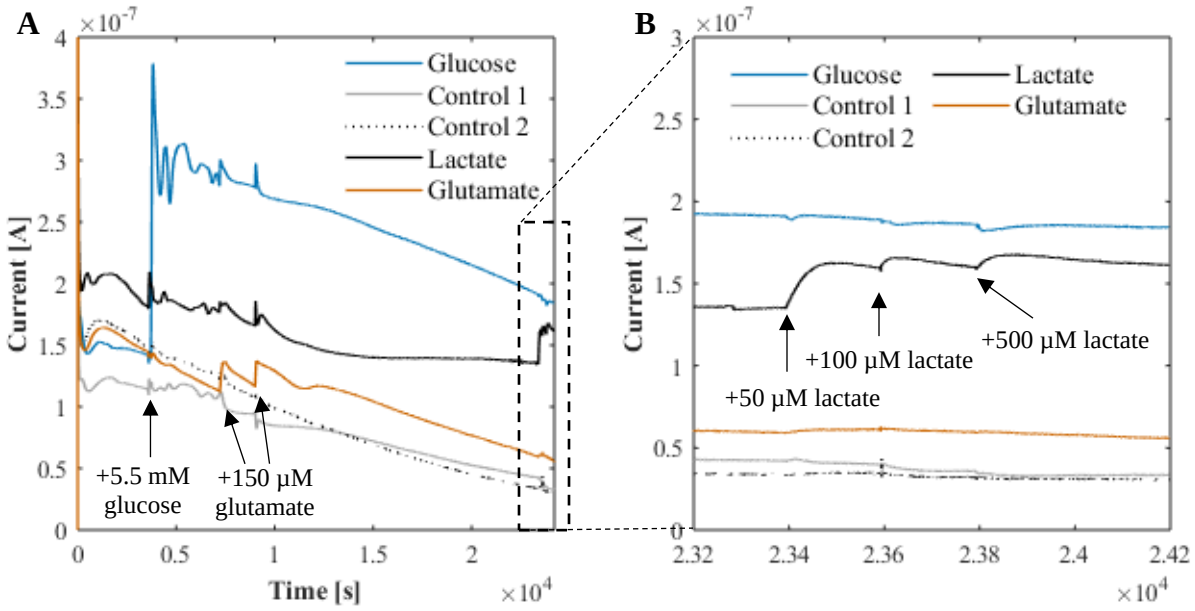

Figure S3. (**A**) Raw current from lactate, glucose and glutamate as a function of time 1 mm above human astrocyte culture in GFDMEM in a humidified incubator at 37ºC, 5% CO_2. Note that these data correspond to **Fig. 5**, except they also include a test of the lactate sensor after measurement (dashed box). (**B**) Inset from 2.32 x 10^4 s to 2.42 x 10^4 s.

REFERENCES

(1) Kissinger, P. T.; Heineman, W. R. Cyclic Voltammetry. *J. Chem. Educ.* **1983**, *60* (9), 702.