

## **A Microfluidic Chip for Sustained Oxygen Gradient Formation in the Intestine *Ex Vivo***

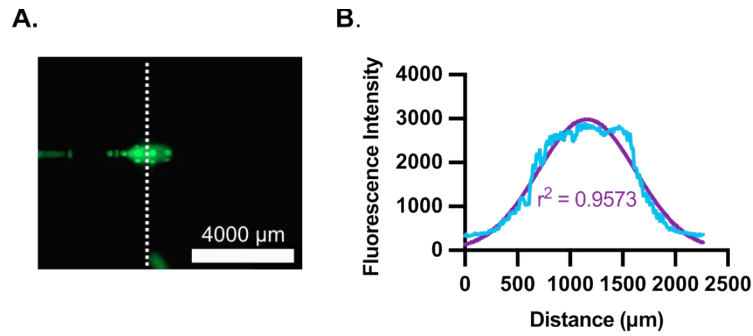
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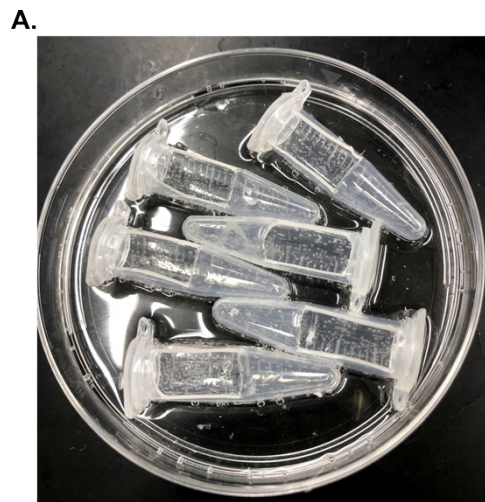
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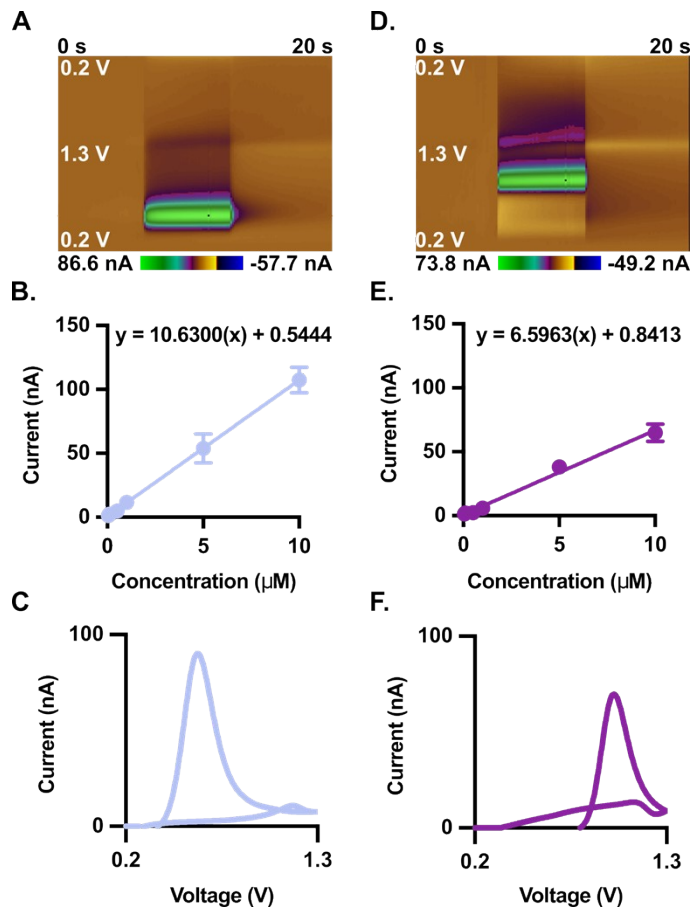
Keywords: tissue culture, microbiome, fast-scan cyclic voltammetry, neuroimmune, carbon-fiber microelectrode



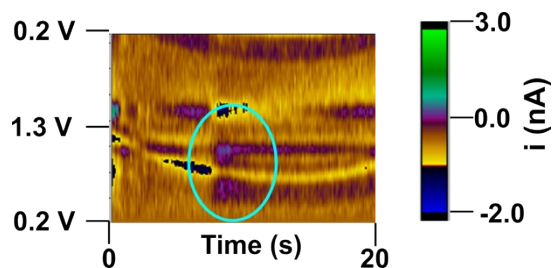
**Figure S1** Gradient size is quantitated on-chip using fluorescence microscopy. (A) Image of fluorescein delivery to an agarose slice on-chip. Gradient was created with a flow rate of 7.5  $\mu\text{L}/\text{min}$  fluorescein and 67.5  $\mu\text{L}/\text{min}$  PBS through the respective channels. Fluorescein media was perfused through the middle “deoxygenated delivery ports” while normal media was delivered through the “oxygenated delivery ports”. (B) Example linescan. Data was taken from the dashed line in A. The spread fits a Gaussian distribution with an  $r^2$  value of 0.9573. To calculate spread, the distance at half maximum intensity was calculated.



**Figure S2** Image of molds for intestinal sections made from 1.5 mL centrifuge tubes



**Figure S3** FSCV provides real-time measurements of serotonin and melatonin in tissue. The “melatonin waveform” was used for all analysis. The waveform scans from 0.2 to 1.3 V at a rate of 600 V/s and frequency of 10 Hz. All measurements were made at a Nafion-coated carbon fiber microelectrode. A) Color plot depicting an example for 1  $\mu\text{M}$  serotonin detection with FSCV. B) Concentration curve for serotonin ( $r^2 = 0.9998$ ). C) Current vs. voltage plot of serotonin. The oxidation peak is observed at 0.7 V. D) Color plot depicting detection of 5  $\mu\text{M}$  melatonin with FSCV. E) A concentration curve for melatonin ( $r^2 = 0.9918$ ). F) Current vs. voltage plot for 5  $\mu\text{M}$  melatonin. Oxidation peak is observed at 1.0 V.



**Figure S4** Representative false color plot for co-detection of serotonin and melatonin in the intestine ex vivo. Potential is on the y-axis, time of data collection is on the x-axis and current is shown in false color. The example transient release of serotonin and melatonin is circled.