

Multiphoton microfabrication of conducting polymer-based biomaterials

John. G. Hardy,^{abc†} Derek Hernandez,^{d†} Damian M. Cummings,^c Frances A. Edwards,^{c*} Jason B. Shear,^{d*} and Christine E. Schmidt^{ab*}

Supplementary Information

Experimental

Multiphoton lithography of polypyrrole

Arrays of polypyrrole wires were fabricated between strips of silver deposited on glass slides *via* multiphoton lithography. A Ti:Sapphire laser at a wavelength of 740 nm and average power of ca. 10 mW (measured at the back aperture of the objective) was focused using a 40X, 0.95 numerical aperture objective and scanned linearly at 115 Hz to initiate the polymerization of pyrrole in chloroform (10 vol % pyrrole). An exposure time of ca. 1 s was used to fabricate the polypyrrole wires. After fabricating the wires, the substrate was washed with ethanol to remove traces of pyrrole and dried under vacuum. The polypyrrole could be doped with camphorsulfonic acid (CSA) during polymerization (stock solution of 0.85 g CSA, 1 mL pyrrole and 9 mL chloroform), followed by washing briefly with ethanol and vacuum drying. Alternatively, the polypyrrole could be doped post-polymerization by reduction of the polypyrrole with sodium borohydride (0.0378 g, 1 mmol) in degassed ultrapure water (18 M Ω , 10 mL) under an inert atmosphere of argon, followed by immersion in a solution of carboxyfluorescein in hexafluoroisopropanol (1 mg/mL) and subsequent oxidation during air drying, followed by rinsing with water and vacuum drying.

Atomic force microscopy (AFM)

An atomic force microscope (AFM, MFP-3D-BIO, Asylum Research) was used to acquire high resolution images of the polypyrrole wires. Images were acquired with the wires both in the dry state and submerged in PBS. Acquisitions were performed in 'contact' mode with a triangular shaped cantilever (silicon nitride, nominal k = 0.03 N/m, MSCCT, Veeco) and a pyramidal tip (2.5 – 8 μ m tall, 40 nm-radius). A 0.5 V deflection setpoint was used to maintain contact during scanning. A scan frequency of 0.5 Hz, scan area of 30x30 μ m, and a 256x256 pixel resolution were used for all images. The height channel was saved as a tab-delimited text file, then imported into Matlab to generate images.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX)

Samples were mounted on metal stubs, coated with Pt/Pd or Carbon using a Cressington 208 benchtop sputter coater before being observed with a Hitachi S5500 SEM equipped with an EDX probe.

Fourier Transform Infrared (FTIR) Spectroscopy

Infrared spectroscopy was carried out on a Thermo Scientific Nicolet 380 FT-IR Spectrometer (Thermo Fisher Scientific Inc., USA). Spectra were recorded in ATR mode at 21 °C, with a 1

cm⁻¹ resolution and 64 scans (corrected for background and atmosphere using OMNIC software provided with the spectrometer).

Conductivity Determination

The conductance of the polypyrrole wires was measured in accordance with protocol IPC-TM-650, number 2.5.17.2 described by the Institute for Interconnecting and Packaging Electronic Circuits. Arrays of wires were examined by chronoamperometry using a CHI900C electrochemical workstation equipped with a picoamp booster and Faraday cage (CHI instruments, Austin, TX). Chronoamperometric measurements were made with a two-point probe system (copper alligator clips), by connecting counter and reference electrodes together. Briefly, two thin strips of adhesive-backed copper tape (Ted Pella, Inc., Redding, CA) were attached to the metal contacts between which the wires were fabricated, separated by a distance of 0.1 cm. The working and counter electrodes were clipped on the strips of copper tape, and the current measured for 50 seconds during a potential step experiment at 10 V. The electrodes were moved to different positions after each measurement, and the current passed was recorded in at least five different positions. The resistance (R , Ω) of the arrays of wires was determined in accordance with equation 1:

$$R = V/I \quad (1)$$

The resistivity (Ω cm⁻¹) of the films was determined in accordance with equation 2:

$$\rho = Rwt/L \quad (2)$$

In which: w corresponds to the width of the individual wires between the metal contacts (corrected for the number fabricated); t corresponds to the thickness of the individual wires in cm; and L corresponds to the length of the wires in cm (0.01 cm). The conductivity (S cm⁻¹) of the polymer was determined in accordance with equation 3:

$$\sigma = 1/\rho \quad (3)$$

Drug delivery

Voltammetry experiments were carried out using a CHI6273C electrochemical analyzer (CH Instruments, Inc.). Electrochemically-triggered release (i.e., de-doping) of carboxyfluorescein from polypyrrole wires using a potential step was achieved by connecting two thin strips of adhesive-backed copper tape (Ted Pella, Inc.) were attached to the metal contacts between which the wires were fabricated, separated by a distance of 0.1 cm. A circle of vacuum grease was drawn around the array of wires to make a well in which 10 μ L phosphate buffered saline (PBS, pH 7.0) could be held. The working and counter electrodes were clipped on the strips of copper tape, and the current measured for 30 seconds during a potential step experiment at + 0.6 V. A potential step of + 0.6 V was applied to the array of wires for 30 seconds, followed by 90 seconds of rest. The concentration of carboxyfluorescein in 2 μ L of solution was quantified by fluorimetry (exciting at 475 nm and reading emission at 520 nm) using a BioTek Synergy HT [®] plate reader (BioTek US, Winooski, VT) equipped with a Take3 Micro-volume Plate and Gen5 v2.04 Software supplied with the plate reader. Samples of 2 μ L were removed from the release medium for each reading (n=5). Readings were corrected by subtracting the reading of PBS alone from each sample. A standard calibration curve for carboxyfluorescein was plotted to define the quantitative relationship between the observed emission and the concentration of carboxyfluorescein. Data plotted are the average of at least three separate trials.

Brain slice preparation and electrical stimulation

Transverse hippocampal brain slices were prepared from a 22 day old C56BL/6j mouse, in accordance with the Animal (Scientific Procedures) Act 1986. Slices of 300 μ m were cut and

bathed in artificial cerebrospinal fluid (ACSF), containing: NaCl 125 mM, KCl 2.4 mM, NaHCO₃ 26 mM, NaH₂PO₄ 1.4 mM, D-Glucose 20 mM, MgCl₂ 1 mM, CaCl₂ 2 mM, ca. 315 mOsm/l, pH 7.4. The stimulating electrode was an array of ca. 150 polypyrrole wires spanning the gap (0.1 cm) between metal contacts to which copper wires were attached with conductive silver epoxy cement. The metal surfaces were coated with waterproof nail varnish (an electrical insulator), and a circle of vacuum grease (also an electrical insulator) was drawn around the array of wires to make a well in which ACSF (50 μL) could be held. The stimulating electrode was positioned to stimulate Shaffer collaterals in the *stratum radiatum*. The recording electrode was pulled from borosilicate glass capillaries (resistance ca. 1-2 MΩ) and was positioned in CA1 region of the *stratum radiatum* to record from CA3-CA1 synapses. A constant potential of 2 V was applied for 100 μs. The field potential was recorded using an AxoClamp2B (Molecular Devices) amplifier and the signal was filtered at 2 kHz prior to digitization (CED Micro1401mkII) and acquisition *via* WinWCP (Strathclyde University). Recordings were carried out using pristine ACSF medium, or ACSF medium containing 1 μM tetrodotoxin (TTX), a potent sodium channel inhibitor.

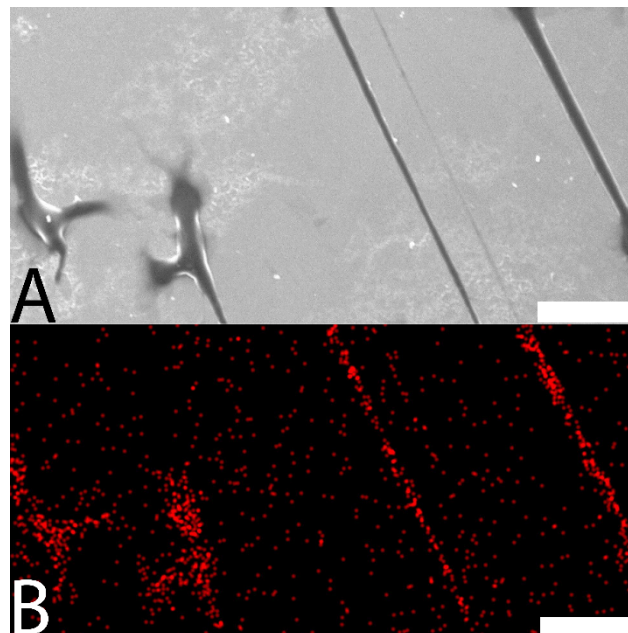


Fig. S1 A and B) SEM images (scale bars represent 20 μm). A) Secondary electron image. B) EDX image, carbon in red. The images highlight the large attachment points on the glass substrate and the long relatively smooth wires that are fabricated from them.

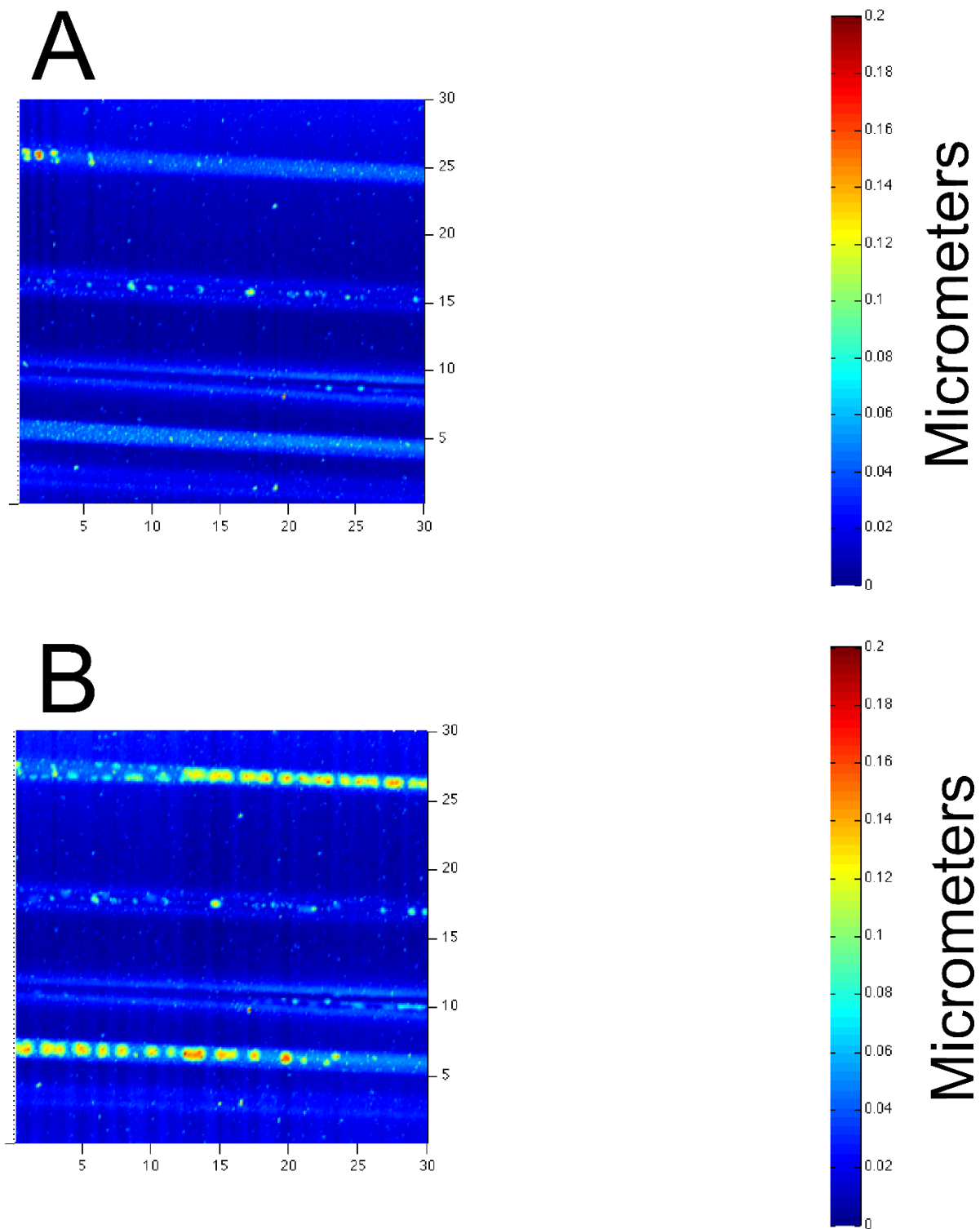


Fig. S2 2D rendering of AFM image of polypyrrole wires. A) dry wires. B) wires swollen in PBS.

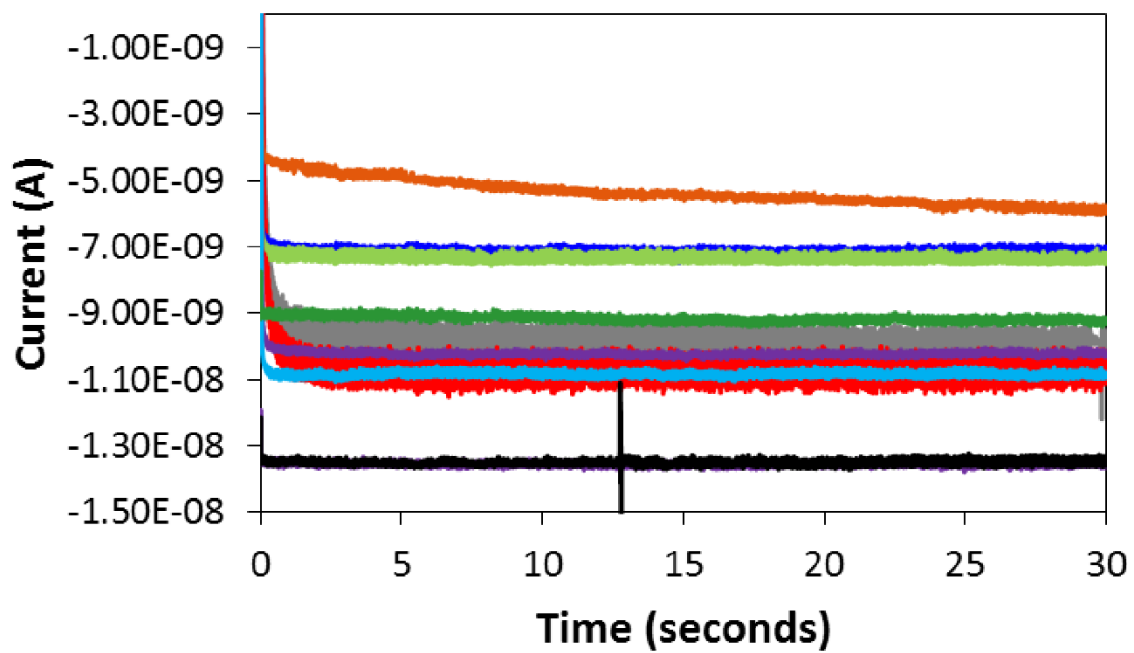


Fig. S3 A sequence of ten chronoamperometric measurements of current stability upon the application of a potential step of 0.6V over 30 polypyrrole wires. 1) grey. 2) black. 3) orange. 4) navy blue. 5) light green. 6) purple. 7) light blue. 8) red. 9) dark green. 10) purple.



Fig. S4 Optical microscope image of an array of black poly(3,4-ethylenedioxythiophene) wires fabricated using an “ink” composed of 3,4-ethylenedioxythiophene (10% v/v) in chloroform. The white scale bar represents 20 μm .