Modular Integration of Hydrogel Neural Interfaces

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Thermal drawing has been recently leveraged to yield multi-functional, fiber-based neural probes at near kilometer length scales. Despite its promise, the widespread adoption of this approach has been impeded by (1) material compatibility requirements and (2) labor-intensive interfacing of functional features to external hardware. Furthermore, in multifunctional fibers, significant volume is occupied by passive polymer cladding that so far has only served structural or electrical insulation purposes. In this letter, we report a rapid, robust, and modular approach to creating multi-functional fiber-based neural interfaces using a solvent evaporation or entrapment driven (SEED) integration process. This process brings together electrical, optical, and microfluidic modalities all encased within a co-polymer comprised of water-soluble poly(ethylene glycol) tethered to water-insoluble poly(urethane) (PU-PEG). We employ these devices for simultaneous optogenetics and electrophysiology, and demonstrate that multi-functional neural probes can be used to deliver cellular cargo with high viability. Upon exposure to water, PU-PEG cladding spontaneously forms a hydrogel, which in addition to enabling integration of modalities, can harbor small molecules and nanomaterials that can be released into local tissue following implantation. We also synthesized a custom nanodroplet forming block polymer and demonstrated that embedding such materials within the hydrogel cladding of our probes enables delivery of hydrophobic small molecules in vitro and in vivo. Our approach widens the chemical toolbox and expands the capabilities of multi-functional neural interfaces.

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Modular integration of hydrogel neural interfaces

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Abstract: Thermal drawing has recently been leveraged to yield multi-functional, fiber-based neural probes at near kilometer length scales. Despite its promise, the widespread adoption of this approach has been impeded by (1) material compatibility requirements and (2) labor-intensive interfacing of functional features to external hardware. Furthermore, in multi-functional fibers, significant volume is occupied by passive polymer cladding that so far has only served structural or electrical insulation purposes. In this letter, we report a rapid, robust, and modular approach to creating multi-functional fiber-based neural interfaces using a solvent evaporation or entrapment-driven (SEED) integration process. This process brings together electrical, optical, and microfluidic modalities all encased within a co-polymer comprised of water-soluble poly(ethylene glycol) tethered to water-insoluble poly(urethane) (PU-PEG). We employ these devices for simultaneous optogenetics and electrophysiology, and demonstrate that multi-functional neural probes can be used to deliver cellular cargo with high viability. Upon exposure to water, PU-PEG cladding spontaneously forms a hydrogel, which in addition to enabling integration of modalities, can harbor small molecules and nanomaterials that can be released into local tissue following implantation. We also synthesized a custom nanodroplet forming block polymer and demonstrated that embedding such materials within the hydrogel cladding of our probes enables delivery of hydrophobic small molecules in vitro and in vivo. Our approach widens the chemical toolbox and expands the capabilities of multi-functional neural interfaces.

Introduction The fiber drawing process enables the fabrication of flexible neural probes that can simultaneously interrogate neuronal circuits via electrical, optical and chemical modalities. During fiber drawing, a macroscopic model (the preform) of the desired probe is fabricated and drawn into hundreds of meters of fibers with microscale features.1,2 To date, these probes have enabled one-step optogenetics,3 in vivo photopharmacology,4 and in-situ electrochemical synthesis of gaseous molecules for neuromodulation.5 Despite these advancements, this approach has several limitations. To be co-drawable, the constituent materials need to have similar glass transition temperatures (for polymers) and melting temperatures (for metals). The resulting melt viscosities must also be compatible to obtain stable draw conditions necessary to maintain the cross-sectional geometry of the preform. Additionally, while thermal drawing yields hundreds of meters of fiber at once, each individual centimeter-long device must be manually connected to back-end hardware, a laborious process that currently represents the fabrication bottleneck. Furthermore, the polymer cladding of these fibers has only served passive structural or electrical insulation purposes, significantly adding to the device footprint with little added functionality.

Hydrogels are an attractive class of materials for neural interfaces.6 The mammalian brain itself is a weak hydrogel with a complex modulus G* on the order of 1 kPa.7 While it has been shown that hydrogels alone can serve as neural interfaces, for example as optical waveguides8 or electrodes,9 their use in multi-functional neural probes has been more limited.10,11 Additionally, while hydrogels have been extensively used as depots for sustained release of bioactive molecules,12 this drug delivery capability has not yet been extended to multi-functional neural interfaces.

In this letter, we complement thermal drawing with a solvent evaporation or entrapment-driven (SEED) integration process to create multi-functional hydrogel-based neural interfaces capable of eluting drugs and nanomaterials. Fibers comprising of optical waveguides, microfluidic channels, or electrode arrays were first drawn individually (Fig. 1A-B). The optical waveguide consisted of a poly(carbonate) (PC) core with a poly(methyl methacrylate) (PMMA) cladding (n_{PC} = 1.586, n_{PMMA} = 1.49), the recording electrode array fiber comprised of four 25 μm tungsten (W) wires within a PC cladding, and the microfluidic channel was a hollow PC fiber. By using a co-polymer that forms a physical gel (PU-PEG) upon exposure to water, we combined these individual modalities into a single physical hydrogel-based neural interface (Fig. 1C-D). This SEED integration process did not require any free radicals or other toxic by-products,13 and could accommodate a wide chemical toolbox previously inaccessible to multi-functional neural probes.
Results and Discussion  To avoid sophisticated cleaning steps associated with potentially toxic radical initiators, we employed a co-polymer of poly(ethylene glycol) tethered to water-insoluble poly(urethane) (PU-PEG), polymers with known biocompatibility routinely used in clinical implants and pharmaceuticals. Upon exposure to water, the PEG blocks facilitate hydration of the material while the hydrophobic forces between PU blocks prevent dissolution, resulting in a physical hydrogel. Since both blocks are soluble in ethanol, we first dissolved the co-polymer in a 95% ethanol solution to form a PU-PEG bath. We then brought the individual fiber components together in this bath, and used a heat source to evaporate the solvent, resulting in an integrated assembly (Fig. 1E).
Figure 2. *In vivo* utilization of the hydrogel neural interface. (A) Image of wake transgenic Thy1-ChR2 mouse chronically implanted with the hydrogel neural interface (top). Image of optogenetically invoking action potentials in anesthetized transgenic Thy1-ChR2 mouse (bottom). (B) Chronic recording of optogenetically-invoked action potentials in the nucleus accumbens (NAc) in a Thy1-ChR2 mouse. (C) Demonstrating release profile of drugs injected through the microfluidic channel using Evans blue dye. Brain atlas image with NAc highlighted in blue (left). Cross-section of a Thy1-ChR2 brain injected with 3 µL of Evans blue dye at 33 nL/s followed by fixation with 4% paraformaldehyde, showing clearly the location of the NAc bolus (middle). Cross-section of the same brain approximately 0.7 mm away, showing the periphery of the depot. (D) Flow cytometry data demonstrating the capability of these neural interfaces to deliver cells with high viability. A mixture of approximately 50% live and 50% dead or dying RAW-Blue murine macrophages (left), and cells injected through the microfluidic channel with the back-fill method at 1 µL/min (middle). We obtained similar viability when comparing to injections with 26G NanoFil syringes and live cells left on ice (right). The histogram was normalized to the mode.
Figure 3. Drug delivery of molecules loaded into the hydrogel itself, independent of the microfluidic channel. (A) Images of hydrogel neural interface loaded with fluorescein implanted into a 0.6% agarose phantom brain over time. (B) Transient release kinetics of fluorescein from integrated fibers with and without PU-PEG. The non-hydrogel control condition was the SEED integration with an equivalent concentration of fluorescein without hydrogel. In addition to depositing a lower concentration, the assembly did not stay integrated without the hydrogel in PBS. Statistical analysis was conducted using two-way ANOVA, \( n = 4 \) in each group, \( ***P = 0.0002 \), \( ****P < 0.0001 \). Error bars shown are ± s.e.m. (C-D) Evans blue dye was loaded into the hydrogel, and this device was implanted into the NAc of Thy1-ChR2 mice. After 72 h, mice were perfused and sections were taken. We imaged these sections with confocal microscopy (4x objective, scans of regions stitched with FluoView software package). (E) Finite element modeling of mass transport of a small molecule from either the microfluidic channel (left) or hydrogel (right) 10 minutes after completion of the 3 \( \mu \)L injection (same time point in both cases). (F) Illustration demonstrating both microfluidic and hydrogel-based drug delivery and the resulting convection vs. diffusion driving forces, respectively.

This integration creates hydrogel fibers that maintain structural integrity upon insertion in a phantom brain model (Fig. S1). We then characterized the electrical, optical, and fluid delivery properties of these hydrogel-integrated probes (Fig. S2-S4). The recording electrodes, \(^2 25 \mu m \)W wires, had impedance of 80 kOhm at 1 kHz which is well within the range suitable for extracellular recordings of neuronal potentials.\(^1\) We chose W over nickel chromium (NiCr) used in tetrodes to avoid gold-plating, which is necessary to achieve sub-MOhm impedance, as that step would expose our hydrogel to an organic solvent.\(^16\) The 25 \( \mu m \)W electrodes were selected over the 12.5 \( \mu m \) alternative because of a > 8-times lower impedance (Fig. S2). Optical losses in the PC/PMMA waveguide were measured as 0.76 dB/cm loss at a 473 nm wavelength, which was consistent with previously observed losses in PC-core fibers and sufficient for optical neural excitation mediated by channelrhodopsin-2 (ChR2) (Fig. S3). We observed an injection efficiency of >90\% for injection rates above 10 nL/s, confirming efficient fluid delivery through the microfluidic channels (Fig. S4). Finally, using dynamic mechanical analysis (DMA), we assessed the bending stiffness of the hydrogel neural interfaces (Fig. S5) and observed that these devices were flexible, in particular compared to other commonly used devices in neuroscience.\(^3,17,18\)

We then evaluated the functionality of our hydrogel-based probes in transgenic mice broadly expressing ChR2 fused to a yellow fluorescent protein under the Thy1 promotor.\(^19\) ChR2 is a light-gated cation channel which, upon irradiation with blue light, causes neuronal depolarization and firing of action potentials.\(^20\) We chronically implanted our neural probes into the nucleus accumbens (NAc) of Thy1-ChR2 mice (Fig. 2A). The NAc plays an important role in the cognitive processing of reward and motivation, and its aberrant function has been implicated in a wide range of mental disorders, including schizophrenia, substance addiction, and post-traumatic stress disorder.\(^21\) The polymer-based optical waveguide and W recording electrodes allowed us to optically stimulate and simultaneously record evoked activity (Fig. 2B; \( \lambda = 473 \text{ nm, 10 Hz, 5 ms pulse width, 20 mW/mm}^2 \)) and spontaneous activity (Fig. S8) of NAc neurons in anesthetized mice. This activity was correlated with laser onset...
due to the photo-electrochemical (Becquerel) effect, To confirm that the observed action potentials were not due to the photo-electrochemical (Becquerel) effect, we also performed electrophysiological recordings during optical stimulation in a chronically implanted Thy1-ChR2 mouse following euthanasia and observed no evoked activity (Fig. S7). We then validated our microfluidic capabilities in vivo by delivering Evans blue dye (2% in sterile saline) into the NAc of Thy1-ChR2 mice. Ten minutes following injection, the animals were transcardially perfused with 4% paraformaldehyde, and widefield microscopy of brain slices revealed a dye depot formation in the NAc (Fig. 2C-D).

Cell-based therapies remain an active area of research for fundamental understanding and future treatments of neurological diseases. We assessed whether our devices are compatible with these emerging therapeutic approaches by evaluating the viability of cells following delivery through the integrated microfluidic channel. Depending on the application, the therapeutic cargo can be front-filled into the tip of the microfluidic channel and delivered during device implantation surgery, or can be back filled days or weeks following chronic implantation (Fig. 2C). We evaluated the viability of RAW-Blue macrophages (RBMs) using both delivery strategies via flow cytometric analysis with DAPI and Annexin V (AnnV) conjugated to Alexa Fluor 647 (AnnV-AF647). As a DNA-binding dye, DAPI was used to probe the viability of cells as fluorescence is only observed when cell membrane integrity is lost during cell death. Annexin V was used to identify exposed aminophospholipid phosphatidylserine (PS). PS is normally maintained on the inner leaflet of the cell membrane under physiological conditions, but becomes exposed during the early stages of regulated cell death and serves as a phagocytic signal. Together, these markers enable quantification of apoptotic and necrotic processes in response to cell stresses or treatments. We applied these markers to compare viability of cells delivered through the microfluidic channel within the hydrogel neural probe to those kept on ice, injected with a 26G NanoFil syringe, or killed via heat shock (Fig. 2D).

With either the front- or back-fill approach, our devices retained RBM cell viability >90% at a 1 µL/min injection rate (Fig. 2E, S9), suggesting that these probes can be used to deliver live cells directly into the central nervous system.

We next demonstrated that the hydrogel cladding enables delivery of small molecules along the entire length of the probe. We used fluorescein as a model drug and co-loaded it into the hydrogel precursor PU-PEG ethanol solution. Since fluorescein is water soluble, introduction into an agarose phantom brain results in diffusion of this molecule away from the hydrogel (Fig. 3A, S10). Quantitative analysis of release into PBS shows a bolus release that peaks 30 minutes post-insertion (Fig. 3B). We then demonstrated this capability in vivo by co-loading Evans blue into the hydrogel and implanting the resulting neural probe into the NAc of Thy1-ChR2 mice (Fig. 3C-D). Confocal microscopy images of brain slices revealed that hydrogel-loaded Evans blue has a different in vivo release profile compared to delivery through the microfluidic channel. Instead of convection-driven transport (first term on the right hand side in Eq. 1) at the tip of the implant, Evans blue delivery is dominated by diffusion-driven transport (second term on the right hand side in Eq. 1) (Fig. 3E-F) and happens along the whole length of the implant. This additional drug delivery modality enabled by the hydrogel may be more advantageous for certain applications, such as the modulation of the foreign body response using anti-fibrotic drugs eluted along the length of implants.

\[
\frac{\partial c}{\partial t} = -\nabla \cdot (cv) + \nabla \cdot (D \nabla c) + R \tag{1}
\]

Controlled drug delivery of hydrophobic small molecule drugs remains a formidable obstacle to their translational utility. Despite recent setbacks, emergent clinical applications of hydrophobic molecules, such as cannabinoids, have garnered renewed interest in their effective delivery. It was recently demonstrated that rationally-designed polymers can overcome the delivery challenges of hydrophobic small molecules by, for example, forming nanodroplets that can carry these molecules into the cytosol. These custom polymers are melts at room temperature, with glass transition temperatures > 150 °C lower than that of PU-PEG, and are not co-drawable with structural polymers typically used in fiber drawing of neural probes. SEED integration allows us to overcome these challenges and thus expands the drug delivery capabilities of neural interfaces. To enable delivery of hydrophobic compounds, a custom block co-polymer (Scheme 1) of PEG and poly(caprolactone) (PCL) was synthesized with an acid-labile ether linkage. This poly(acetal), named PA11 (1:1 ratio of PEG:PCL), was blended with the PU-PEG precursor solution at a 3:17 ratio, and the resulting blend was applied during integration of a fiber assembly. We found that PA11 releases from the PU-PEG matrix and forms nanodroplets approximately 25 nm in diameter in saline solution under mild perturbations (Fig. 4A-C). As a positive control, we repeated the experiment with a thick film of the PU-PEG/PA11 blend and vigorously shook it overnight, which resulted in PA11 nanodroplets of similar size. Transmission electron microscopy (TEM) images of the dehydrated samples further corroborated formation and dimensions of these nanodroplets (Fig. 4B). We then assessed the ability of PA11 to deliver hydrophobic compounds into aqueous media. We found that when the hydrophobic small molecule Nile Red was mixed in with the PA11:PU-PEG composite, PA11 could escape and carry the hydrophobic dye with it (Fig. 4C). By immersing Nile Red-loaded hydrogel fibers with and without PA11 into PBS and measuring the fluorescence after gentle shaking overnight, we observed release of the hydrophobic small molecule only when PA11 was co-loaded into the hydrogel (Fig. 4D-E). To further illustrate this functionality in the context of neurobiology, we incubated primary rat dorsal root ganglion (DRGs; sensory neuronal structures) with Nile Red in the presence or absence of PA11 (Fig. 4F, S11). We found that after a 24 h incubation with PA11, 96% of neurons were Nile Red-positive (Fig. 4F). No dye was found in neuronal cytoplasm or nuclei in the absence of PA11 (Fig. 4G, S11), suggesting this polymer is sufficient for effective intra-neuronal delivery of hydrophobic small molecule drugs. Finally, we extended this concept in vivo and demonstrated that tissue along an implant contained Nile Red 72 h after an implantation of a PA11/Nile Red-loaded hydrogel probe (Fig. 4H-I).

**Conclusions** By leveraging a SEED integration approach that employs amphiphilic co-polymers, we created modular hydrogel neural interfaces capable of optogenetics, electrophysiology, and microfluidic delivery. We demonstrated that these devices are capable of delivering a variety of cargo, including cellular therapies with a high viability...
Figure 4. Drug delivery of nanoparticles and hydrophobic small molecules loaded into the hydrogel. (A) Dynamic light scattering (DLS) data of the solution after elution of PA11 from PA11/PU-PEG-based fibers. The vial containing the fiber was gently perturbed over 24 h. As a positive control, we vigorously shook a thick PA11/PU-PEG film in PBS. (B) Transmission electron microscopy (TEM) images of PA11 nanodroplets. (C) Supernatant from a film of PU-PEG loaded with Nile Red without (top) or with (bottom) PA11. (D) Quantification via fluorescence spectroscopy of Nile Red loaded into hydrogel neural interfaces with and without PA11. Statistical analysis was conducted using ordinary one-way ANOVA. n = 3 in each group, ***P < 0.001 (P = 0.0003 vs. saline, P = 0.0004 vs. -PA11). Error bars shown are ± s.e.m. (E) A full fluorescence spectra of the fiber supernatant when both Nile Red and PA11 are incorporated. Excitation sweep was obtained with a fixed emission wavelength of 640 nm (black). Emission sweep was obtained with a fixed excitation wavelength of 550 nm. Arbitrary unit axes were rescaled for clarity. (F) Confocal micrographs (60x) of primary rat dorsal root ganglion neurons (DRGs) co-incubated with media containing both PA11 and Nile Red for 24 h. (G) Quantification of intensity on the Nile Red channel compared to no PA11 control. Statistical analysis was conducted using unpaired t test. n = 4 in each group, ***P = 0.0001. (H-I) in vivo demonstration of NR/PA11 elution in the NAc of C57BL/6 mice after 72 h at (H) 4X and (I) 60X magnification.

at fast injection rates. By loading model drugs or nanomaterials into the hydrogel itself, we also demonstrated a separate drug delivery modality with a unique driving force and release profile. We further showed the potential to extend this concept to new directions, including drug delivery of hydrophobic cargo. This platform expands the chemical toolbox and functionability available to soft neural interfaces.

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Ethics The authors declare no conflicts of interest. All animal experiments in this work were approved by the MIT Committee on Animal Care.

Supporting Information Electronic supplementary information (ESI) is available for this work. ESI contains experimental details and Supporting Figures S1-S11.
Modular integration of hydrogel neural interfaces

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S.1 Materials and Methods

Chemicals and Reagents
Poly(carbonate) and poly(methyl methacrylate) rods or tubes were purchased from McMaster-Carr. Tungsten and nickel chromium wires were purchased from Goodfellow. Poly(urethane)-poly(ethylene glycol) was purchased from AdvanSource Biomaterials Corp (D3). Poly(ethylene glycol) (M.W. = 400 Da), poly(ε-caprolactone) (M.W. = 530 Da), tri(ethylene glycol) divinyl ether (TGDVE), para-toluenesulfonic acid monohydrate, tetrahydrofuran (pTSA), acetone, ammonium hydroxide, fluorescein sodium salt, Nile Red, hexamethyldiamine (solid and 70% solution), Evans blue dye, iron (III) chloride hexahydrate (ACS Reagent, 97%), poly(vinyl alcohol) (87-89% hydrolyzed), chloroform (ACS reagent ≥ 99.8%), 1-octadecene (≥ 91%), hexane (ReagentPlus, ≥ 99%), benzyl ether (98%), oleic acid (90%), sodium chloride (≥ 99%), potassium chloride (≥ 99%), magnesium chloride hexahydrate (≥ 99%), calcium chloride dihydrate (≥ 99%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES,
≥ 99.5%), D-glucose (≥ 99.5%), 4-morpholineethane sulfonic acid hydrate (MES hydrate, ≥ 99.5%), sodium hydroxide (ACS Reagent, ≥ 97%), DMSO, and amiloride hydrochloride hydrate (≥ 98%) were purchased from Sigma Aldrich. Capsaicin and sodium oleate were purchased from TCI Chemicals. Matrigel solution was purchased from Corning. Neurobasal medium and serum-free B27 were purchased from Gibco. Fluo-4 AM, Trypsin-EDTA (0.25%), and fetal bovine serum were purchased from Invitrogen. Dulbecco’s Modified Eagle’s Medium (DMEM, high glucose) was purchased from ATCC. Murine RAW-Blue macrophages were purchased from InvivoGen. DAPI (4’,6-Diamidino-2-phenylindole, di-lactate; 422801), Alexa Fluor 647 Annexin V (640912), and Annexin V binding buffer (422201) were obtained from BioLegend.

Animals
In this study, 7 to 10-week-old, male and female Thy1-ChR2-YFP mice (generously donated by Prof. Guoping Feng) and C57BL/6 (Jackson Laboratory) were used. Mice were housed at the MIT animal facility, and regular housing conditions (12 h L/D cycle, 22 °C, food and water ad libitum) were used. For primary culture, male and female Sprague-Dawley rat pups (P0) were used. All experiments were approved by the MIT Committee on Animal Care.

Thermal Fiber Drawing
The neural probe was fabricated by integrating individual fiber probes into a hydrogel cladding to form a single multifunctional device. Each modality (electrical, optical and microfluidic) was fabricated by using the thermal drawing process on macroscopic templates (preform) of the desired fibers. The optical waveguide was fabricated by inserting a 3/8 in polycarbonate rod (PC, McMaster) into a 3/8 in ID x 1/2 in OD poly(methyl methacrylate) tube (PMMA, UsPlastic 44020), which was consolidated under vacuum at 100 °C for 15 min, then drawn at 220 °C to achieve a 200 micron fiber. The microfluidic channel was
fabricated by machining a 5/8 in PC rod (McMaster), then drawing it at 220 °C to achieve a 200 micron fiber with a 90 micron lumen. The electronic fiber was fabricated by milling four channels along a PC rod, then rolling PC films along the preform to cover the holes from the edge. The preform was then consolidated under vacuum at 175 °C before being co-drawn with 25 micron tungsten wire (GoodFellow) at 220 °C to achieve the 120 micron fiber. All three drawing were performed using a custom-built fiber drawing tower.[1]

**Connectorization**

Each modality was connectorized separately. The optical waveguide was bonded to an optical ferrule (Thorlabs) with UV epoxy and polished as previously reported[1]. The microfluidic channel was bonded to tubing with UV epoxy. The PC cladding of the tungsten tetrodes was scraped with a steel blade and each wire was individually soldered to electronic pins. A steel screw and stainless steel wire were soldered to the 5th pin which served as the ground wire.[1, 2]

**Hydrogel Integration Tower**

A stepper motor attached to a stage was purchased and connected to an Arduino. The stepper motor speed was calibrated and set to 1 mm/s. An extending clip was 3D printed and tightly attached to the stage to extend its size and provide a substrate to hold the neural interface. We added double-sided tape to the stage, and this was used to hold the neural interface in place during the integration process. A 3 mL syringe was cut and loaded with the hydrogel precursor solution. The twisted assembly (see Fig. 1) was inserted and removed from the bath before heating, resulting in a 20 ± 7 µm cladding.

**Characterization measurements**

Electrical impedance was measured with a precision LCR meter (HP4284A, Agilent) with a sinusoidal input (10 mV, 10²-10⁶ Hz). Optical transmission loss was calculated by mea-
suring laser output through the waveguide for different fiber lengths with a photodetector (Thorlabs S121C, 400–1100 nm range). Microfluidic characterization was conducted by connecting the microfluidic tubing to a NanoFil syringe and injecting 9 \( \mu \)L of DI water into a sealed phantom brain (0.6% agarose) at various injection speeds and measuring the weight of the vial post-injection. Delamination measurements were conducted by measuring the distance between the center of the three modalities via cross-section of the tip before and after insertion into a 0.6% agarose phantom brain (1 cm, approximately 1 mm/min insertion). XPS measurements of amine-functionalized PC fibers were conducted on a X-ray Photoelectron Spectrometer (Versaprobe II). The bending stiffness was measured with a dynamic mechanical analyzer (Q800, TA Instruments). Samples 1 cm in length were mounted with a cantilever clamp at 37 °C and measured with a frequency sweep (0.01–10 Hz) under controlled 50 micron displacement.

**Implantation, optogenetics and electrophysiology**

All animal experiments were approved by the MIT Committee on Animal Care. Surgeries were performed on deeply anesthetized mice (isoflurane) and positioned in a stereotactic frame (David Kopf Instruments). Fibers were implanted into the nucleus accumbens (NAc) with the following coordinates: 0.75 mm anterior-posterior, 1.25 mm mediolateral, -3.9 mm dorsoventral. Electrophysiological recordings were conducted with a RZ5D recording system through a PZ2-32 headstage (Tucker Davis Technologies). A screw attached to the skull was used as a ground. Optogenetic stimulation was achieved by using a 500 mW, 473 nm diode-pumped solid-state laser (OEM Laser Systems) coupled to a ferrule connected to the polymer waveguide. Stimulation was done at 20 mW/mm\(^2\) with a 10 Hz frequency and 5 ms pulse width.

**Drug release experiments**

Fluorescein release experiments were conducted by loading PU-PEG with the small molecule
and integrating. Fibers were cut to 1 cm and placed in 5 mL of PBS. Before the intensity was measured with a plate reader at each time point, the vials were gently shaken to homogenize the solution. For Nile Red measurements, PA11/Nile Red mixtures (5 mg NR/100 µL of PA11) were mixed with 10% PU-PEG at a 3 to 17 ratio and the devices were integrated. Release was conducted in PBS at room temperature and the same procedure was followed prior to plate reader measurements as for fluorescein.

**Finite element modeling**

Mass transport was modeled with COMSOL Multiphysics finite element package (5.3a). Convection or diffusion-driven transport (Fig. 3C) were modeled in water. The built-in properties of water were used. The brain hemisphere geometry was approximated as a 3 by 6 mm rectangle. A 3 mm long rectangle was used to approximate the fiber, and a 90 micron lumen was used as the inner diameter of the microfluidic channel. We set a no flux outer boundary condition and utilized a diffusivity of $10^{-7}$ cm$^2$/s.

**PA11 synthesis and imaging**

PA11, a block co-polymer of PEG and PCL connected with biodegradable ether linkages, was synthesized as reported elsewhere[3]. Briefly, PEG, PCL-diol, and TGDVVE were added in a 1:1:2 molar ratio in dried THF under nitrogen (total concentration 0.667 g/mL). pTSA (0.5 wt%) was added to the stirring reaction mixture. After 2 h, ammonium hydroxide was added to quench the polycondensation. The solvent was removed with rotary evaporation and the polymer was dialyzed (3.5 kDa cutoff). The solvent was again removed with rotary evaporation then under vacuum. PA11 was imaged using transmission electron microscopy. A nano-droplet solution obtained by vortexing PA11 in water was deposited to a carbon-coated copper grid and dried. The polymer was imaged using a transmission electron microscope (Tecnai-F20, FEI Company). Dynamic light scattering measurements of nano-droplets in PBS were conducted on a Malvern Nanoseries Zeta-
sizer at room temperature (refractive index of 1.7).

**RAW-Blue cell culture**

NF-κB reporter RAW 264.7 murine macrophage cells (RAW-Blue, Invivogen) previously frozen at passage 6 were thawed and cultured in DMEM supplemented with 10% fetal bovine serum. After reaching 70-80% confluency, cells were collected and passaged using cell scrapers. Subcultures were seeded at manufacturer's recommended concentration (1.5x10⁴ cells/cm²) and Zeocin antibiotic (200 /µg/mL) was added during every other passage to provide selection pressure to the cell line. Cells were used at passage 9 for flow cytometry experiments.

**Primary neuronal culture**

Matrigel was mixed with Neurobasal media (with B27 supplement) in a 1:30 ratio and left overnight. Glass coverslips (12 mm) were coated with 70 µL of this solution. Whole DRGs (P0, Sprague-Dawley rat pups) were seeded on the coverslips and incubated in 1 mL of Neurobasal/B27 media. Primary neurons were kept in a cell culture incubator at 37 °C and 5% CO₂.

**Fixation and confocal microscopy**

Animals were anesthetized with isoflurane, injected with fatal plus (100 mg/kg IP), and transcardially perfused with 50 mL of ice cold phosphate buffered saline (PBS) followed by 50 mL of ice cold 4% paraformaldehyde (PFA) in PBS. The devices were carefully explanted and the brains were removed and fixed in 4% PFA in PBS for 24 h at 4 °C. Confocal microscopy images were obtained using a laser scanning confocal microscope (FluoView FV1000, Olympus). Brain slices were dried and mounted using Fluoromount. 4x, 20x, and 60x objectives were used for imaging.
S.2 Supporting Figures

Figure S1: We measured the distance between the center of the three modalities within the fiber before and after insertion into a 0.6% agarose phantom brain and observed no statistically significant changes. The sum of the distance between the modalities is plotted before and after insertion.

Figure S2: Electrical impedance characterization of hydrogel fibers with various recording electrodes. We chose the 25 micron tungsten (W) wire due to its low impedance (approximately 80 kOhm) at 1 kHz frequency.
Figure S3: Waveguide optical loss characterization. Light intensity was measured at different lengths. The loss was calculated to be 0.76 dB/cm.

Figure S4: Microfluidic characterization of hydrogel neural interfaces. Output injection rate and injection efficiency of the microfluidic channel of 2cm long fibers were calculated by injecting 9 µL of DI water into a phantom brain (0.6% agarose) at an injection speed of 1, 10, 25, 50 and 100 nL/s (n = 4). Volume injected was measured by weight.
Figure S5: We measured the bending stiffness of our devices using dynamic mechanical analysis (DMA) and observed they were soft compared to other commonly used tools in neuroscience, such as silica waveguides[2].

Figure S6: Controls experiment for optically-evoked electrophysiological activity using the neural implant implanted in the NAc of a Thy1-ChR2 mouse. Comparison of electrophysiological response to 10 Hz yellow LED light (595 nm, 10 ms, 20 mW/mm^2, 10H), 10 Hz, 100 Hz, and prolonged pulse Blue LED light (470 nm, 10 ms, 20 mW/mm^2). Only blue irradiation elicits electrophysiological activity (A-B). 10 Hz stimuli elicits a correlated activity (B, top) that is stable and reproducible over every trials (N=10) as seen on the associated raster plot (middle) and peri-stimulus time histogram (bottom). 100 Hz (C) and a single prolonged pulse (D), elicits decaying and uncorrelated activity.
**Figure S7:** Progressive extinction of optically-evoked electrophysiological recording in a Thy1-ChR2 mouse after euthanasia (t=0; left). Expanded plots at A, B, and C are included (right) and show the progressive loss of evoked activity after approximately 1.5 min, thus corroborating the physiological nature and not photovoltaic artifact of the recorded activity.

**Figure S8:** We recorded endogenous neuronal activity from the hydrogel neural interface at 1 wk in the NAc (top). Zoomed in plots (bottom) illustrate the spike waveform.
Figure S9: Flow cytometry results of RBMs. After subjecting cells to delivery methods, they were collected and stained in Annexin V binding buffer at 1:100 AnnV-AF647 and 3 $\mu$M DAPI for at least 15 minutes. Cells were then washed in Annexin V buffer, prepared as single cell suspensions by passing through cell strainer caps of FACS tubes, and analyzed on BD LSR-II. (A) Flow scatter plot of cells kept on ice (left), injected through a 26G NanoFill needle at 1 $\mu$L/min (right), or cells flown through the hydrogel neural interface with one-step loading[2] at 1 $\mu$L/min (right). (B) Quantification of % live, necrotic, apoptotic, and dead cells. All conditions had high (>90% viability). We observed back-fill loading resulted in a slightly higher cell viability than one-step loading.

Figure S10: Time-resolved images from a FITC-loaded PU-PEG hydrogel neural interface inserted into a 0.6% agarose phantom brain.
Figure S11: Confocal microscopy images of DRGs incubated with Nile Red without (top) and with (bottom) PA11 after 24 h. Cells were washed with PBS and fixed with 4% PFA before imaging.

References

(1)  A. Canales, X. Jia, U. P. Froriep, R. A. Koppes, C. M. Tringides, J. Selvidge, C. Lu, C. Hou, L. Wei, Y. Fink and P. Anikeeva, *Nature Biotechnology*, 2015, **33**, 277–284.

(2)  S. Park, Y. Guo, X. Jia, H. K. Choe, B. Grena, J. Kang, J. Park, C. Lu, A. Canales, R. Chen, Y. S. Yim, G. B. Choi, Y. Fink and P. Anikeeva, *Nature Neuroscience*, 2017, **20**, 612–619.

(3)  L. Zhang, Z. Zhang, W. Wang, A. Tabet, S. Hanson, L. Zhang, D. Zhu and C. Wang, *ACS Applied Bio Materials*, 2021, DOI: 10.1021/acsabm.1c00194.
