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Electronic Supplementary Information (ESI)

Surface modification of neural electrodes with pyrrole-hyaluronic acid conjugate to attenuate reactive astrogliosis in vivo†

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Materials and Methods

Conjugation of pyrrole to hyaluronic acid

1-aminopropyl pyrrole was first synthesized as previously described[1, 2]. Then, PyHA was prepared by dissolving 200 mg of HA (1.6×10^6 Da, Fluka) in 200 mL of double de-ionized (DDI) water overnight, followed by the addition of 198 mg of 1-ethyl-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide (EDC, Sigma-Aldrich), 116 mg of N-hydroxysuccinimide (NHS, Sigma-Aldrich), and 0.15 mL of 1-aminopropyl pyrrole. The pH of the solution was adjusted to 5.5 using 0.2 M HCl. After stirring for 20 h, the solution was dialyzed (10,000 Da molecular weight cut-off, Spectrum Labs) against DDI water at room temperature for 3 days, exchanging the water every day. The solution was freeze-dried and the solid was stored at -20°C. PyHA was characterized using 1H NMR. The degree of pyrrole addition was 5-15%, which was calculated based on 1H-NMR from the ratio of the relative peak integrations of the pyrrole protons (peaks at δ 6.1~6.7) and the acetyl protons on the backbone of HA (peak at δ ~1.9).

Surface modification of neural probes with poly(PyHA) via electrochemical polymerization

Commercial neural probes were electrochemically coated with PyHA: (1) microwire probes (Plexon), and (2) silicon microelectrode probes (A1x8-4mm-200-1250, NeuroNexus Inc). The silicon microelectrode probe consists of a 4 mm-long single shank with 8 iridium electrodes (1250 μm2 each electrode) on a silicon nitrite insulating pad. The microwire electrode is a 4 mm-long single tapered iridium microwire of 100 μm in diameter. Electrochemical deposition of poly(PyHA) was performed using a solution of PyHA (5 mg/mL) in DDI water. Potential cycling between 0 and 1.0 V, versus a standard calomel electrode (SCE), was employed at a scan rate of 0.1 V/s. Twenty cycles were applied for each substrate using a computer-assisted potentiostat (Electrochemical Analyzer CHI6273C, CH Instruments) with a three-electrode

configuration, in which a neural electrode, a Pt mesh, and a SCE electrode served as a working electrode, a counter electrode, and a reference electrode, respectively. After the coating, the probes were washed extensively with sterile DDI water.

Characterization of poly(PyHA)-coated neural probes

The poly(PyHA)-coated neural probes were characterized by staining of HA using HA binding protein[1] and electrochemical impedance measurements. For HA staining, the substrates were incubated in phosphate-buffered saline (PBS) (50 mM, pH 7.2) containing biotinylated-hyaluronic acid binding protein (bHABP; 1:200) (Calbiochem) for 1 h, washed twice with PBS, treated with streptavidin-phycoerythrin (streptavidin-PE; 1:500) (Molecular Probes) in PBS for 1 h, and washed with PBS three times. Fluorescence and phase contrast images were acquired using a fluorescence microscope (IX-70, Olympus) equipped with a color CCD camera (Optronics MagnaFire). Electrochemical impedance spectra from the modified and unmodified electrodes were acquired from an Electrochemical Analyzer (CH Instruments) in a frequency range of 1-10^5 Hz, by applying an AC sinusoidal signal of 10 mV in PBS (50 mM, pH 7.2).

Probe implantation

All animal work was performed in accordance with the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin. The poly(PyHA)-coated probes and unmodified probes (controls) were sterilized by UV exposure for 2 h prior to animal surgery. The surgical procedure was performed under sterile conditions. Ten male Sprague-Dawley rats (Charles Rivers), weighing 300-350 g, were anesthetized with 2-3% isoflurane (Abbott) inhalation in an induction chamber. General anesthesia was maintained at 2% isoflurane, and breathing speed and depth was monitored throughout the experiment. The hair on the head was shaved and the animal was transferred to a stereotactic frame with continuous delivery of isoflurane during the surgical procedure. A small mid-line incision of the skull was made

along the rostrocaudal line using aseptic surgical techniques. The exposed skull was carefully cleaned and dried. Two small holes (approximately 1 mm diameter) were drilled 1.6 mm anterior to bregma and 3 mm from the midline on each side. Bone debris was removed and the dura mater was broken using a 23-gauge needle (BD Bioseciences). Either the microwire electrode or the silicon microelectrode probe was inserted into the motor cortex through an opening in the skull on each hemisphere of the cortex. Two different probes (microwire and silicon microelectrode) either unmodified or modified were implanted into each animal; four animals were implanted with unmodified probes and the five animals were implanted with poly(PyHA)–modified probes. Dental acrylate and super glue were successively added and cured around the holes with the electrodes, and the skin was sutured using 6.0 Ethilon suture. The animals were treated with buprenorphine (3.0 mg/kg, Carpuject®, Hospira Inc.) to alleviate post-surgical pain. The animals were allowed to recover fully on a warm pad, and then transferred to their home cage.

Perfusion and tissue sample preparation

Three weeks after implantation, the animals were anesthetized using intraperioneal injection of an overdose of ketamine (100 mg/mL, Bioniche epharma) and xylazine (20 mg/mL, Anased®, Lloyd Laboratories). The animals were perfused transcardially first with ice cold PBS (~200 mL, MP Biomedicals), and 4% paraformaldehyde (200 mL, Sigma-Aldrich) in PBS, followed by decapitation. The head was post-fixed in a fresh fixing solution (4% paraformaldehyde in PBS) at 4ºC overnight and transferred to 30% sucrose solution in PBS containing 1% Kathon (Supelco) at 4ºC for several days. Brains were dissected from the skull. Intact probes were pulled out carefully from the brains under a surgical microscope and stored in sterile PBS until analyzed. Brain samples were sectioned coronally (50 μm thick) using a cryotome, and freely-floating brain sections were stored at -80°C in a cryo-protected buffer (20% sucrose solution) until use.

Immunofluorescence

Brain sections were incubated in PBS containing 0.5% Triton X-100 (Sigma) and 3% goat serum (Sigma-Aldrich) in PBS at room temperature for 2 h. The sections were incubated in an antibody solution of rabbit anti-GFAP (1:1000, Abcam) at 4ºC overnight. Then, the samples were washed three times with PBS (15 min each), followed by the secondary antibody treatment with a solution of Alexa Fluor 568-labeled goat anti-rabbit antibody (1:500) at room temperature for 2 h. After three washes with PBS (15 min each), the brain samples were counter-stained with DAPI, and mounted onto coverslips (Corning) with Fluoromount-G solution (Southern Biotech).

Imaging and analysis

Immunofluorescence images were acquired as described above using a fluorescence microscope (IX-70, Olympus) equipped with a color CCD camera (Optronics MagnaFire). Notably, all samples from all animals were simultaneously processed and handled in the same way. Images were acquired using the same exposure time on a single day. Fluorescence images were analyzed to evaluate tissue responses. In particular, sections 1.5-1.8 mm deep from the surface of the brain were analyzed using ImageJ software (NIH). The same threshold value was applied for all samples to obtain binary images responding to a specific marker. The area that was positively stained by the marker was measured. From each group, average and standard error of the means (SEM) were calculated and reported.

References

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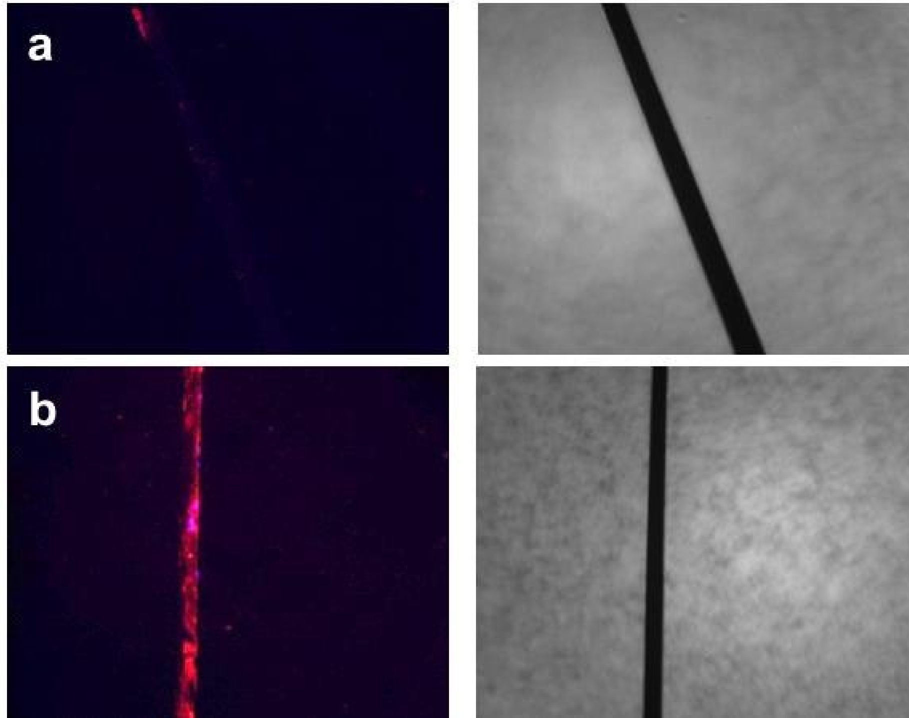

Fig S1. Representative immunofluorescence (left) and bright-field (right) images of the retrieved microwires implanted in rat brain cortices for 3 weeks. (a) Unmodified control microwires and (b) the poly(PyHA)-coated microwires were retrieved from the fixed brains and stained using bHABP.

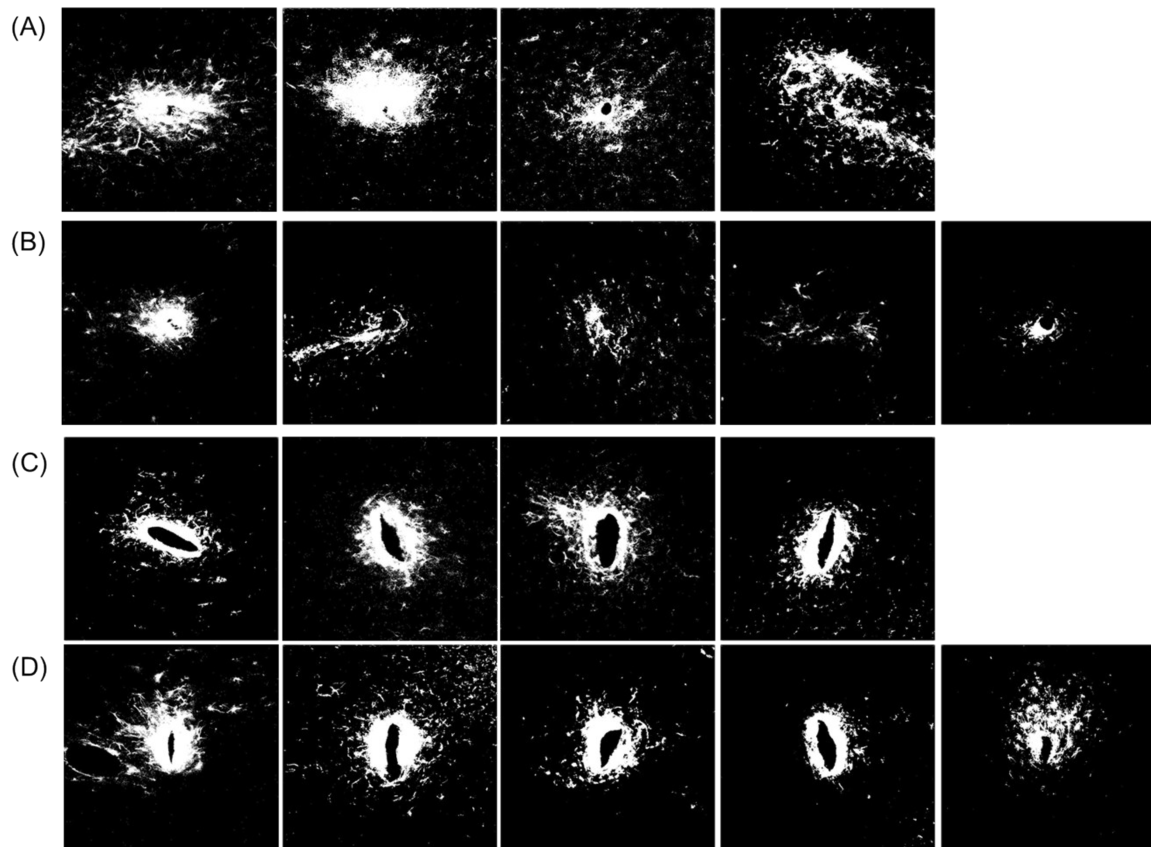

Fig S2. Representative GFAP immunofluorescence images of brain sections from the animals implanted with (A) unmodified microwires, (B) modified microwires, (C) unmodified silicon microelectrode probes, and (D) modified silicone microelectrode probes.